1944/45

A CONTRIBUTION TO THE KNOWLEDGE OF THE ACID POLYURONIDES

BY

SIGURD SÄVERBORN

Printed in Sweden by
Almqvist & Wiksells Boktryckeri AB
Uppsala 1945

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INAUGURAL DISSERTATION

BY

SIGURD SÄVERBORN
FIL. LIC.

BY DUE PERMISSION OF THE PHILOSOPHICAL FACULTY OF THE UNIVERSITY OF UPPSALA, TO BE PUBLICLY DISCUSSED IN THE LECTURE ROOM OF THE CHEMICAL INSTITUTE, ON MAY 9TH, 1945, AT 10 A. M.

UPPSALA 1945
ALMQVIST & WIKSELLS BOKTRYCKERI AB

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581/48

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PREFACE.

This work has been carried out at the Institute of Physical Chemistry at the University of Uppsala during the years 1941—1944, and should be regarded as a link in the series of ultracentrifugal investigations of high molecular carbohydrates which have been going on at the Institute for several years.

To the head of the Institute, Professor The Svedberg, who suggested this investigation, I wish to express my deep gratitude for his never-failing interest in my efforts and for his encouraging kindness. I am also grateful for his personal friendship.

To my colleagues at the Institute I am much indebted for valuable discussions and helpful advice.

I should like to thank Mr. Evald Hellman and his staff for carrying out numerous calculations.

Part of the expenses connected with this work were defrayed by grants from the Rockefeller Foundation and the Wallenberg Foundation. Financial support was also given by Kärnbolaget AB, Stockholm.

The author wishes to express his thanks to Mrs. William Cameron for the revision of the English text, and to the British Council for making a grant to cover the expenses for this revision.

Uppsala, March 1945.

Sigurd Säverborn.

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INTRODUCTION.

The high molecular carbohydrates or polysaccharides in a wider sense may be divided according to their composition into two large groups, viz. simple polysaccharides and complex polysaccharides (cf., for instance, 1). The former are built up of only one kind of sugar, the latter of two or more sugars or sugar derivatives. Cellulose, starch, inulin, for instance, are simple polysaccharides, pectic substances, gums, mucilages and many of the substances termed hemicelluloses are complex polysaccharides.

Analyses of the complex polysaccharides have shown that most of them contain uronic acid units, linked to simple sugars. It was suggested by Candlin and Schryver (2) that substances built up in this way should be termed *polyuronides*. This term will be made use of in the present work.

The polyuronides to be found among the hemicelluloses are in an exceptional position in so far as they show no acidic properties, despite the presence of uronic acid units. No satisfactory explanation of this peculiar fact has hitherto been given (3). The other polyuronides, such as pectic substances, gums etc., exhibit acidic properties, or may easily be converted into acids, owing to the fact that they occur *in situ* as esters or salts.

In the present work three types of acidic polyuronides, viz. *pectic substances*, *alginic acid* and *gum arabic* have been investigated, mainly from a physico-chemical point of view. The complex nature of these substances has made it difficult for chemists to establish their composition and constitution, and to decide whether they are on the whole to be regarded as chemical entities. The fact that they are colloidal electrolytes has added to the difficulties met with in the determination of several physico-chemical constants.

The *pectic substances* have been more extensively investigated than any of the other polyuronides. The history of these substances goes back to the year 1790. In that year Vauquelin (4) published a work entitled *Analyse du Tamarin* where it was emphasized for the first time that the jellying power of certain fruit juices must be ascribed

to a specific substance, or, to use the words of the author, 'un principe gelatineux'. This jellying substance was isolated and investigated in the early 1820's by Braconnot (5), who named it *pectin* in order to emphasize its most distinguishing property.

In the 1840's Frémy (6) succeded in showing that pectin is to be found in unripe fruits in an insoluble form which he called *pectose*. This name has subsequently been changed for the more suitable one of *protopectin* at the suggestion of Tschirch (7). Frémy followed both microscopically and otherwise the course of the ripening process, and was able to show that, during this process, the protopectin is transformed into soluble pectin. A similar transformation may be achieved by boiling the unripe fruit for some time in water or dilute acid.

Frémy also studied soluble pectin and its decomposition products, assigning to all of them names and exact formulas, at which he had arrived by analysing the salts of the acid substances with heavy metals. Pectin, for instance, was given the formula $C_{64}H_{96}O_{64}$.

Analyses of a similar kind and calculations of formulas for the pectic substances were made by many of Frémy's famous contemporaries, such as Mulder, Regnault, Chodnew and Berzelius, in the belief that the composition of these substances could be defined by simple formulas.

The proper method of attacking the constitutional problems was indicated by Scheibler's (8) discovery in 1868, which showed that hydrolysis of beet pectin with dilute sulphuric acid results in the formation of arabinose. It seemed probable that this simple sugar was a unit in the pectin molecule, and the search for others could begin.

In 1889, Wohl and von Niessen (9) were also able to show that galactose, or some derivative of this sugar must be a constituent of pectin as oxidation of pectic substances with nitric acid gives large amounts of mucic acid. This fact was also confirmed by Herzfeld (10) in 1890.

In 1918, von Fellenberg (11) published the results of his extensive investigations on the occurrence of methyl alcohol in different foodstuffs, and pointed out that methyl groups are to be found as a constituent in the pectic substances. This fact made him devote his attention to the constitution of these substances. He was able to show that the percentage of methyl alcohol in the pectic substances investigated varied between 9 and 11·5, that the methyl groups occur in ester formation and that the solubility in water of the substances depends on the degree of esterification.

In 1917, Ehrlich (12) and Suarez (13) announced independently the discovery of *d*-galacturonic acid, and Ehrlich proved it to be a constituent making up the major part of all pectic substances.

From this fact and the results of several analyses of pectic substances obtained from apples, oranges, currants, quinces and sugar beet, von Fellenberg proposed the hypothetical formula

$$C_{62}H_{96}O_{52}(COO \cdot CH_3)_n \cdot (COOH)_{8-n},$$

to represent the composition of the pectin molecule. This formula includes two arabinose units, one unit each of methyl-pentose and galactose, and eight galacturonic acid units, partially esterified with methyl groups, i. e. not less than five different kinds of units. The methyl-pentose unit has not been mentioned in later investigations. The completely demethoxylated substance ($n = 0$) was called *pectic acid*.

The discovery of *d*-galacturonic acid was made by Ehrlich in connection with his investigations of beet pectin. The results of these investigations, as interpreted by Ehrlich (14) himself, have been until recently the basis of the theory of the constitution of the pectic substances, and are still quoted in many textbooks and books of reference. Ehrlich called protopectin 'Urpektin' or 'Genuines Pektin'. On boiling with water this is converted to 'Hydratopektin', which may be separated into an araban component, soluble in 70 per cent alcohol, and the Ca-Mg salt of 'Pektinsäure', which is transformed by acids into 'Primäre Pektinsäure'. This acid, according to Ehrlich, is a well-defined substance, having the definite formula $C_{41}H_{60}O_{36}$ irrespective of the origin of the pectic material. Cryoscopic molecular weight determinations performed by Ehrlich and Schubert (15) gave values of the order of magnitude demanded by the formula. On hydrolysis the substance is split into four *d*-galacturonic acid units, two units each of methyl alcohol and acetic acid and one unit each of arabinose and galactose. It thus contains methoxyl groups and ought to be distinguished from the pectic acid defined by von Fellenberg. Ehrlich considered the four galacturonic acid units to exist in the pectic substances as a four-membered ring. To this tetragalacturonic acid was ascribed the jellying power of the pectins.

The presence of acetyl groups in the pectic substances is first mentioned by Smolénski (16). According to him the framework of pectin is a polygalacturonic acid of chain structure, the units being linked to each other glycosidically. The structural pattern would thus

be that attributed by Haworth to cellulose. Nanji, Paton and Ling (17), on the other hand, were of the opinion that pectin could be represented by a six-membered ring composed of four galacturonic acid units and one unit each of arabinose and galactose.

During the last decade, however, most scientists agree that the basal unit of pectic substances is built up in the manner suggested by Smolénski. By methylation of citrus pectic acid (in the sense of von Fellenberg), Morell, Baur and Link (18) isolated fragments of the substance consisting of eight to ten galacturonic acid units; a fact evidenced by 'end group' determinations and by cryoscopic molecular weight determinations. Viscosity measurements and X-ray analysis pointed to the existence of a straight chain structure. — Baur and Link (19) investigated the products which Ehrlich defined as tetragalacturonic acid, and showed them to be composed of seven to ten galacturonic acid units. — From a large amount of analytical data, Myers and Baker (20) came to the conclusion that the nucleus of the pectin molecule must be an octagalacturonic acid.

During the years 1936—1938 most important works were published by Schneider and co-workers. They strongly emphasize that the pectins are built up as chain molecules and that the degree of polymerization is far greater than had been hitherto supposed. — Henglein and Schneider (21) call attention to the inconsistency between the views held by Ehrlich and the evidence obtained from X-ray analyses by Meyer and Mark (22), Corbeau (23) and others. They stress that the properties of pectins and methyl cellulose are in many respects such as to indicate a similar structure. Esterification of pectins with nitrate, acetyl and formyl groups was used in order to obtain products soluble in organic solvents such as acetone, amyl acetate etc. In this way the disturbing effects of the charges were eliminated and viscometric and osmotic molecular weight determinations made possible.

Schneider and Fritschi (24) came to the conclusion that 'nitro' pectin contains no arabinose, galactose or any acetyl groups, but is made up exclusively of partially esterified galacturonic acid units.

Finally Schneider and Bock (25) discuss the constitution of the natural (water soluble) pectins and show the untenability of the formula proposed by Ehrlich for his pectic acid. They doubt strongly whether arabinose and galactose are constituents of the pectin molecule, and emphasize the possibility of misinterpretation consequent on the fact that pectin may be mixed with araban and galactan. The common method

of precipitating the pectin with alcohol results in precipitation of the high molecular araban and galactan as well, and special measures will have to be taken in order to get rid of them. The araban, for instance, may be broken down by hydrolysis with dilute acids, a procedure which does not affect the pectin. Araban and galactan may be extracted by hot 70 per cent alcohol (cf. 27) etc. In any case, the authors suggest that pectin may be identified with a polygalacturonic acid, esterified to a certain degree with methyl groups, as shown by the formula in Fig. 1.

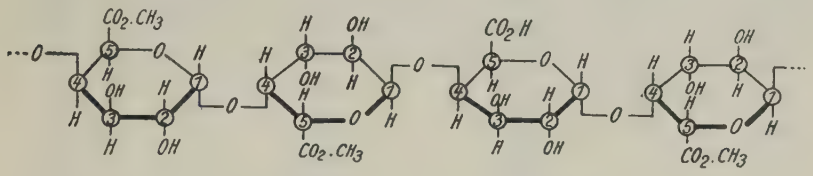

Fig. 1.—Structure of the essential part of the pectin molecule. (Elsner, 26.)

This view is supported by Hirst and Jones (27) who were able to isolate an araban from peanut pectin and a mixture of araban and galactan from apple pectin. On the other hand, evidence will be given later on pointing out the necessity of assuming in addition units other than galacturonic acid, as constituents of the pectin molecule.

The structure of the citrus polygalacturonide has been studied by Luckett and Smith (28). These authors arrived at the conclusion that a 1·4 α-glucosidic structure of pyranose units is the most probable one.

On the basis of the ideas developed in recent years as to the structure of pectin, Henglein (29) has outlined a hypothetical pattern for *protopectin* as shown in Fig. 2. According to this diagram protopectin is composed of polyglacaturonide chains linked together by means of Ca (Mg or Fe) atoms. It would also be possible for a polygalacturonide chain to be linked to a cellulose chain, as the cellulose molecule contains a few scattered glucuronic acid units.

The characterization of the pectic substances from a physico-chemical point of view has been largely confined to data concerning viscosity and jellying power under different conditions. Molecular weight determinations have very rarely been recorded. The figures given have been obtained in most cases by pure calculation based on analyses of pectin. Molecular weights of the magnitude 2 000, 1 100 and 1 900

Fig. 2.—A hypothetic pattern for protopectin. (Henglein, 29.)

respectively can be calculated from the formulas proposed by von Fellenberg and Ehrlich (p. 9) and by Myers and Baker (*l. c.* p. 10). As has already been mentioned, cryoscopic determinations were carried out by Ehrlich and Schubert, and by Morell, Baur and Link. In both cases values of the order of magnitude 1 300 were obtained. It was stated by the latter scientists, however, that the materials investigated were fragments of pectin, and this must also have been the case in the determinations described by Ehrlich and Schubert.

Probable values for the molecular size of the native pectins from apple and sugar beet were obtained for the first time by Schneider and co-workers (*l. c.*) from their osmotic and viscometric measurements on nitro pectin in acetone. Values of the order of 20 000—200 000 were obtained, a quite different magnitude, therefore, than that mentioned above.

From measurements of the sedimentation and diffusion velocity of pectin in the juice of apples, pears, plums and oranges Svedberg and Gralén (30) reported molecular weights of the order 25 000—50 000.

By the same method the present author (31) found values of the order 50 000—100 000 for pectins extracted from apples and citrus albedo.

Evidence of the polydispersity of pectin was first given by Mehlitz (32) in 1927. He succeeded by means of ultra-filtration in separating particles of different sizes. Thus one third of the particles in a sample of apple pectin were longer than 0·6 μ, while the rest had dimensions of about 0·2—0·6 μ.

Alginic acid is a cell wall constituent similar to pectin. It occurs in certain marine algae, e. g. various species of *Laminaria*, *Ascophyllum* and *Fucus*, some of which may contain as much as 40 per cent of it (36). It was discovered by Stanford (33) in 1883.

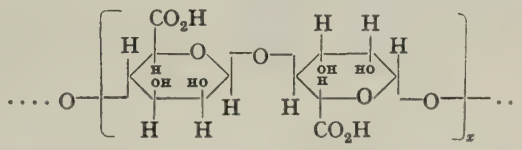

Fig. 3.—Structure of alginic acid. (Hirst, 39.

The constitution of this polyuronide has been elucidated chiefly through investigations performed by Nelson and Cretcher (34), Dillon and McGuinness (35), Lunde, Heen and Öy (36), Hirst and Jones (37), and Lucas and Stewart (38). It is probable that alginic acid is made up almost exclusively of *mannuronic acid* units in the pyranose form, linked together 1·4 β-glycosidically (Fig. 3). Alginic acid and pectic acid would thus be isomers.

The state of the acid *in situ* is not clear. Part of it is combined as a Ca, Mg salt, but no simple esterification occurs. nevertheless the tissues are not acidic.

A determination of the molecular weight of alginic acid is described by Heen (40). Using 2 M-NaOH as a solvent he obtained from viscosity measurements an average of about 15 000, corresponding to a degree of polymerization of about 80. He emphasizes, however, that the values must be considered as approximations only, since it is uncertain whether Staudinger's formula can be applied.

Gum arabic is not a normal cell-wall constituent. It is formed by a pathological process and exuded from fissures or wounds in the bark of various species of *Acacia* found in the Tropics.

Through O'Sullivan's (41) pioneer investigations in the 1880—1890's it was shown that gum arabic and most other gums are built up according to a general pattern, including an acid nucleus to which is attached readily hydrolysable sugars. Butler and Cretcher (42) showed that the acidic properties are due to the presence of uronic acid units. Further investigations made by Challinor, Haworth and Hirst (43), Smith (44) and Jackson and Smith (45) have made it possible to outline the main features of a repeating unit in the molecule. From Fig. 4 it may be seen that glycuronic acid, galactose and rhamnose units in the pyranose form, and arabinose units in the furanose form, are the

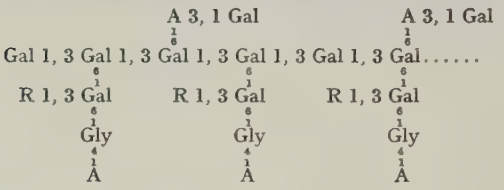

Fig. 4.—Possible arrangement of the constituents in the repeating unit of the gum arabic molecule. (Hirst, 39.

constituents of gum arabic. The prevalence of 1·3 and 1·6 linkages and the occurrence of branched chains is to be noted. There is thus a marked structural difference between this polyuronide and the other two. This difference also manifests itself in the physico-chemical properties.

Gum arabic occurs *in situ* as a Ca, Mg salt.

Investigations of the particle size or molecular weight of gum arabic seem to have been published so far only by one author. In connection with the presentation of a new type of osmometer for low pressures Oakley (46) gives some preliminary figures which are supplemented in a later paper (47). Values of the order of 200 000—290 000 were obtained for the particle size, the magnitude depending on the nature and concentration of the electrolyte used for the depression of the charge effects.

At concentrations lower than about 0·3 per cent, the osmotic pressure was too small (1—2·5 mm water) to be accurately measured and Oakley suggests measurements with the ultracentrifuge as the only means for obtaining accurate data at such low concentrations.[1]

[1] It might be of interest to note here that measurements of very low osmotic pressures were recently described by Jullander and Svedberg [Nature *153*, 523 (1944)]. By using an 'osmotic balance' they succeeded in measuring pressures of the magnitude 0·7 mm of water with an accuracy of about 0·03 mm.

The main object of the present investigation was to elucidate the behaviour of the acid polyuronides on sedimentation and diffusion and to make an attempt to establish their average molecular weight.

Owing to their complex nature, however, these substances require some kind of preliminary definition in each particular case. The first chapters are thus devoted to details regarding preparation and composition. In addition, some physico-chemical properties of particular interest were dealt with before entering upon the various problems connected with sedimentation and diffusion.

PREPARATION OF MATERIALS.

Most of the substances investigated in this work have been prepared in the laboratory. Numerous methods of preparation have been described in the literature on the subject, especially in the case of pectic substances. Some of them have been made use of here. The procedure will be described briefly.

PECTIC SUBSTANCES.

Pectins which are to be found in the juice from fruits or vegetable materials, or which may be leached out in water without boiling will be called *free pectin* (cf. Ripa, 48, p. 123), those which have been obtained by boiling the vegetable materials in water, dilute acids, or some salt solution, will be called *extracted pectins*.

A. Free Pectins.

1. From apples. — Fresh, ripe apples (*Cox pomona, Cox orange*) were pared, cored and pressed, and the juice obtained filtered down into twice its volume of 96 per cent alcohol. The precipitated pectin was filtered off, pressed, dissolved in water and precipitated a second time.

In order to remove ash constituents from the pectin preparations the solutions were usually dialysed against 0·05 N-HCl over night and afterwards against distilled water until the chloride ions had disappeared, i. e. after one or two days with frequent changes of water. In this way the ash content was reduced from 3 à 4 per cent in many cases to about 0·1 per cent.

2. From red currants. — The juice was decolorized by means of activated carbon and treated as just described.

3. From lemon albedo (Ripa, 48, p. 124). — The yellow layer of the peel of fresh lemons was removed, the albedo peeled off, chopped into pieces and leached out in water of 30° C for twenty-four hours. The extract was treated as in 1.

B. Extracted Pectins.

1. Apple pectin (von Fellenberg 11, p. 136). — The fruit flesh was cut into slices and boiled out in 96 per cent alcohol four times, 20 minutes each time, to remove

colouring matter or other substances soluble in alcohol which might interfere with the pectin. After the alcohol was squeezed out, the pectin was extracted by boiling the material in water for one hour. The pectin was precipitated by adding to the extract twice its volume of alcohol acidified with 7 ml hydrochloric acid per litre.

2. Commercial apple pectin, produced by Unipektin A.-G. Zürich. — The method of preparation is not known in this case but the high jellying power (jelly grade 320, i. e. one part of pectin will produce a satisfactory jelly with 320 parts of sugar and the proper amounts of water and acid) of the substance is a good guarantee that no drastic methods have been used. — The pectin was purified by re-precipitation. The investigation of this substance was of a special interest owing to its great practical value.

3. Citrus pectin (Myers and Baker, 49, p. 54). — Lemon peels were minced and leached in alcohol at room temperature for one week to remove resins, tannins, terpenes and glycosides. The material was then dried and ground to a rather fine powder. This was further extracted with acetone in a Soxhlet apparatus to remove residues of colouring matter. — 50 grams of this albedo were added to 3 litres of a boiling citric acid solution of pH 2·15 (13·15 grams of crystallized citric acid per litre) and the boiling continued for half an hour. The mixture was then cooled rapidly to room temperature, the extract clarified and the pectin precipitated with alcohol. It was further washed successively with alcohol, acetone, and ether, and then dried in air.

4. Beet pectin.[1] — Soaked chips of sugar beet were treated in the way described in the preceeding paragraph, except that the materials were allowed to stand over night in the citric acid solution to facilitate the liberation of the pectin at the subsequent extraction.

5. Carrot pectin. — Fresh carrots were minced and pressed, and the solid material treated as the lemon peels described above.

6. Flax pectin (Lüdtke and Felser, 50, p. 21). — The starting material was so called 'Grünflachsstaub', i. e. pieces of the bast tissue which are removed from the flax fibres on swingling, heckling etc. — The materials were freed from wax and fat by extraction with chloroform — for want of dichlormethane, the solvent recommended by Lüdtke and Felser — in a Soxhlet apparatus, and further treated with 0·5 per cent hydrochloric acid to remove ash constituents, washed free of the acid and gently boiled for 30 minutes in 0·5 per cent ammonium oxalate solution. The pectin was recovered from the extract by precipitation in acidified alcohol.

The pectin thus obtained is called A-pectin as distinguished from another pectic substance occurring in the flax stem which is called B-pectin. The latter is very firmly attached to the flax fibres and cannot be extracted without resorting to rather drastic means.

C. Pectic Acid.

5 grams of commercial apple pectin (B 2) were dissolved in 500 ml of 0·1 N-NaOH by stirring for 10 minutes at room temperature. The temperature was then slowly

[1] The author is indebted to Mr. O. Wiklund, Chief Chemist at Svenska Sockerfabriks Aktiebolaget, Arlöv, for supplying sugar-beets and chips.

raised to 40° C, the solution filtered and poured slowly into an excess of hydrochloric acid. The gelatinous pectic acid precipitate was filtered off, dehydrated and washed free of chlorides with alcohol, rinsed with acetone and ether and dried in air.

ALGINIC ACID.

1. Mainly according to Lunde, Heen and Öy (36, p. 199). Fresh 'leaves' of *Laminaria digitata*[1] were minced and left to stand over night in 0·5 per cent hydrochloric acid. The materials were then washed free of acid with cold water and treated twice with boiling hot water. The alginic acid was extracted at room temperature with 0·5 per cent sodium carbonate solution, precipitated from the clarified extract with hydrochloric acid and a preliminary purification made by repeated precipitations from a solution in 0·4 per cent sodium carbonate.

Further purification was achieved by dissolving the substance in 0·1 N-sodium carbonate, dialysing the solution for twenty-four hours against the solvent and subsequently against distilled water until sodium alginate was chiefly left in the cellophane bag. To the sodium alginate solution was added dilute hydrochloric acid under vigorous stirring and the precipitated alginic acid was separated and further treated as described above for pectic acid.

2. Commercial sodium alginate, produced by A/S Protan, Drammen, from *Laminaria digitata*.

GUM ARABIC.

The raw materials were obtained from a chemist's shop. To judge from external characteristics such as form, colour, and above all the pattern of cracks — these characteristics are closely discussed in Wiesner, Die Rohstoffe des Pflanzenreiches (4. Aufl. 1927) — the gum probably belonged to some kind of Nile gum and was thus of good quality.

The substance was purified by acidifying a 20 per cent aqueous solution with hydrochloric acid and precipitating the gum in three volumes of alcohol. After three such precipitations the gum was dissolved in water, and the solution dialysed against distilled water until the chloride ions had disappeared, i. e. after about one or two days with frequent changes of water. The ash content was thus easily diminished from about 3 per cent to about 0·05 per cent.

Some of the substances enumerated above have been selected as *type substances* and subjected to closer investigation than the others. The substances chosen are commercial apple pectin (B 2), citrus pectin (B 3), beet pectin (B 4), alginic acid (II: 1) and gum arabic.

[1] The author is indebted to Dr. G. Gustafson, Director of the Zoological Station at Kristineberg, for supplying various species of algae.

SOME ANALYTICAL DATA.

The percentage uronic acid anhydride and methoxyl of the polyuronide preparations are to be found in Tables 1 and 2. Table 2 refers to the type substances and contains in addition some other characteristic data.

The following notes may be added to the various points of the tables.

TABLE 1. — COMPOSITION OF THE POLYURONIDE PREPARATIONS.

Preparation	Uronic acid anhydride, per cent	Methoxyl content, per cent
Apple pectin, free	72·2	9·2
Citrus pectin, »	80·2	12·0
Red currant pectin, free	68·4	8·7
Apple pectin according to v. Fellenberg's method	69·3	10·5
Carrot pectin	65·7	7·4
Flax pectin	76·0	2·3
Pectic acid from commercial apple pectin	93·3	0·3
Alginic acid, commercial	96·2	—

TABLE 2. — DATA REFERRING TO THE TYPE SUBSTANCES.

Properties	Apple pectin	Citrus pectin	Beet pectin	Alginic acid	Gum arabic
Uronic acid anhydride, per cent	85·4	83·6	71·3	91·0	17·8
Methoxyl content, per cent	8·4	11·6	10·2	—	—
Degree of esterification, per cent	55·8	78·8	81·2	—	—
Equivalent weight of the acids (de-esterified pectins):					
a) From carboxyl content	198	199	236	192	989
b) From titration	195	199	184	190	1200—1300
$[\alpha]_D^{20}$ (c = 1—2 per cent)	+247	+230	+196	−139	−28

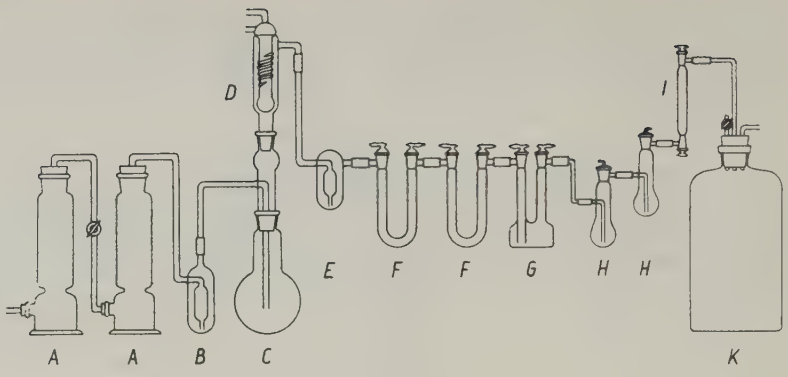

Fig. 5.—Apparatus for the quantitative determination of the uronic acid constituent of the polyuronides, according to the Lefèvre-Tollens method.

The sample to be analyzed (in the case of pectins and alginic acid about 0·5 g) and 100 ml of 12 (or 18, cf. text) per cent hydrochloric acid are placed in the 250 ml reaction flask *C*, the apparatus is connected to a water pump through the safety bottle *K* of 5-litre capacity and air is aspirated through the system. On passing the two 20-cm soda lime towers *A* and the bottle *B*, which contains concentrated potassium hydroxide, the air is freed from carbon dioxide. After the absorption tubes *H*, containing ascarite backed by dehydrite, have attained constant weight the reaction flask *C* with content is heated in a glycerine bath to 140° C, this temperature being maintained for eight hours, while air is constantly drawn through the system. *D* is a reflux condenser, *E* a silver nitrate trap for removing traces of hydrochloric acid and *F* U-tubes, containing concentrated sulphuric acid, distributed by means of glass beads, for removing water vapour and interfering decomposition products, any remaining water being taken up in the drying tube *G*, which is filled with dehydrite. *I* is a protecting tube.

Uronic Acid Anhydride.

The percentage uronic acid anhydride was determined by a modified Lefèvre and Tollens' method (51). The method depends upon decarboxylation of the uronic acid units by distillation with 12 per cent hydrochloric acid under a reflux condenser and absorption of the carbon dioxide produced. The apparatus employed may be seen in Fig. 5. In the case of alginic acid it was found necessary to use 18 per cent hydrochloric acid in order to attain complete decarboxylation.

The percentage figures in Tables 1 and 2 refer to the unit $C_6H_8O_6$ as the polymerizing unit. They are calculated from the ratio $C_6H_8O_6/CO_2 = 4$.

Methoxyl Content.

The CH_3O-groups were determined by the modified Zeisel method described by Vieböck and Schwappach (52), consideration being given to the additions made by Neumann (53).

Equivalent Weight of the Polyacids.

The titrimetric determination of the equivalent weight of pectic acid was performed by dissolving about 0·2 g of pectin in 40—50 ml

of 0·1 N-NaOH in an Erlenmeyer flask provided with a ground glass stopper, and leaving the flask in a thermostat at 50° C for three hours in order to secure complete demethoxylation. An excess of 0·1 N-HCl was then added and back titration performed with 0·1 N-NaOH, using brom thymol blue as the indicator. — The quantity of pectic acid corresponding to a given quantity of pectin is obtained by multiplying the weight of the pectin by $(1 - 0{\cdot}004516 \cdot p)$, p denoting the percentage methoxyl.

Alginic acid and gum arabic were treated with standard alkali at room temperature.

The values calculated from the carboxyl content and those obtained from titration agree fairly well in the case of apple pectin, citrus pectin and alginic acid. The discrepancy between the two values for beet pectin may be attributed to the probable occurrence of acetyl groups in the molecule, cf. Nelson (54).[1] The corresponding discrepancy for gum arabic is probably due to the fact that some of the carboxyl groups occur under ordinary conditions in lactone formation, cf. Butler and Cretcher (42).

The equivalent weight values are of a certain interest from a constitutional point of view. If the pectic substances and alginic acid are to be considered as pure polyuronides, i. e. built up exclusively from uronic acid units, the equivalent weight of the acids would be equal to 176. The formula proposed by Schneider (p. 11) requires this value. Schneider himself gives no data about the equivalent weight, however, and the value 176 has never been given in the literature on the subject. Olsen and co-workers (55) arrived at the value 201 as the average equivalent weight of pectic acid from apples and citrus fruits and refer to Sloep who found the value 205, and to Bonner, who used several different methods, and obtained figures ranging from 201 to 207. Some figures reported by Hirst (56) in 1942 are reproduced in Table 3.

In any case, pectic acid from rather varied sources seems to have

[1] After this was written, it was shown by B. Eriksson at this institute, using the method of Freudenberg-Harder (Hans Meyer, Analyse und Konstitutionsermittlung, 5. Aufl., 349, 1931) that a sample of beet pectin, prepared according to the description on p. 17, contained 4·2 per cent acetyl groups, while apple and citrus pectin contained no such groups. Taking this fact into account, the titrimetrically determined equivalent weight of beet pectin would be equal to 192, or close to that actually determined, provided the acetyl groups were really split off.

Table 3. — Equivalent Weight of Pectic Acid.

Source	Equiv. wt.
Strawberry	200
Raspberry	187
Apple	195
Citrus	215
Lupinus albus, seeds . .	210

an equivalent weight equal to about 200. The figures of Table 2 for apple and citrus pectic acid agree well with this average. The discrepancy between the experimental values of the equivalent weight and the theoretical value 176 makes it difficult to accept wholly the formula given by Schneider. Olsen (55) agrees with Bonner (57) that pectic acid may be considered as made up of long chains of galacturonic acid units 'with an occasional galactose and arabinose unit scattered along the chain'.

The values reported for the equivalent weight of alginic acid exhibit rather large variations, with an average of about 190 (cf. 3, p. 145). Dillon and McGuinness (35) point to the possibility that the polymerizing unit of the acid is not the uronic anhydride but the complete mannuronic acid group itself, having an equivalent weight equal to 194. Lunde, Heen and Öy (36) do not agree with this interpretation, and analyses made by them strongly support the view that the uronic acid unit is of the anhydride type.

The equivalent weight of various samples of gum arabic has been found to vary from about 1 000 to about 1 400.

From the percentage uronic acid anhydride and methoxyl given in Table 2, the composition of the type pectins may be outlined as follows.

	$(C_5H_7O_4COOCH_3)_m$	$(C_5H_7O_4COOH)_n$	R
Apple pectin	51·5	37·7	10·8 per cent
Citrus pectin	71·1	17·7	11.2 »
Beet pectin	62·4	13·5	24·1 » »

R denoting a residue of unknown composition. According to Schneider and Bock (25) R may possibly be some substance mixed with the

partially esterified polygalacturonic acid. In order to find out if this was the case, the apple and citrus pectins were precipitated from their aqueous solutions, once in 60 per cent alcohol and a second time in 50 per cent alcohol, as described by the authors mentioned. The pectins were furthermore treated with 70 per cent alcohol on the water bath (65—70° C) for four hours, a method suggested by Hirst and Jones (27) for removing pentosans. After this treatment the following figures were obtained for the composition of the pectins.

	$(C_5H_7O_4COOCH_3)_m$	$(C_5H_7O_4COOH)_n$	R
Apple pection	51·6	38·8	9·6 per cent
Citrus pectin	70·5	20·5	9·0 » »

There is, indeed, a small decrease in R, but on the whole the figures speak in favour of R being part of the molecule. It may be made up of those scattered galactose and arabinose units mentioned by Bonner. This view is also confirmed by the fact that attempts to separate the uncharged and supposedly free R fraction from the charged remainder by electrophoresis have never succeeded. Each of the three pectins migrated as a whole (cf. p. 47).[1]

In the case of alginic acid, some 9 per cent of the substance may similarly be accounted for by non-uronic material.

[1] In the case of flax pectin, again, B. Eriksson (unpubl.) succeeded in obtaining electrophoretically a fraction containing about 90 per cent uronic acid anhydride, while the starting material contained only 76 per cent.

VISCOSITY.

The viscosity of solutions of the type substances is illustrated by the data given in Tables 4—8 and Figs. 6—7. The measurements were made with Ostwald viscometers having a capillary length of about

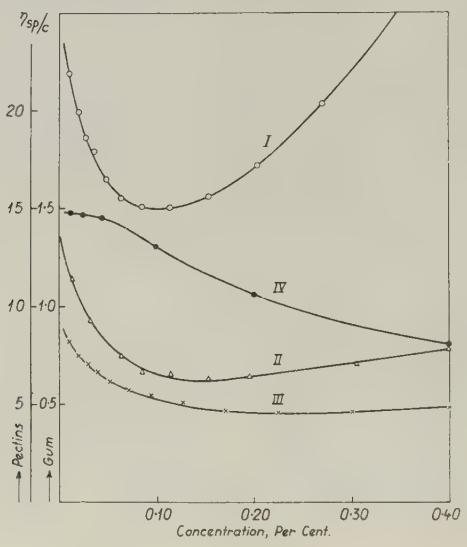

Fig. 6.—η_{sp}/c for the polyuronide type substances in pure water, as a function of concentration.
I. Citrus pectin. II. Commercial apple dectin. III. Beet pectin. IV. Gum arabic.

10 cm and times of outflow for water at 20° C ranging from 32 to 125 seconds. η_r indicates relative viscosity, i. e. the ratio of times of outflow for solution and solvent, $\eta_{sp} = \eta_r - 1$ specific viscosity. The concentration c is given in weight per cent.[1]

[1] Staudinger and co-workers usually give the concentration of solutions of high polymers in number of mols of the polymerizing unit per litre of solution, c_{gm} ('Grundmolarität'). Denoting the molecular weight of the polymerizing unit by m, $c_{gm} = 10\ c/m$ for dilute aqueous solutions. The polymerizing unit of the pectins dealt with here has an average molecular weight equal to 183 if the non-uronic units are assumed to be $C_6H_{10}O_5$. For alginic acid the corresponding value is 175.

TABLE 4. — CITRUS PECTIN, VISCOSITY AT 20° C.

Medium:	H_2O		0·2 N-NaCl			
Conc. wt. per cent	η_r	η_{sp}/c	Conc. wt. per cent	η_r	log η_r	η_{sp}/c
0·480	18·51	36·48	0·300	6·809	0·8331	19·36
0·360	10·33	25·92	0·225	4·666	0·6690	16·29
0·270	6·47	20·26	0·169	3·229	0·5091	13·19
0·203	4·473	17·11	0·127	2·435	0·3865	11·30
0·152	3·374	15·62	0·095	1·942	0·2882	9·92
0·114	2·708	14·98	0·071	1·641	0·2149	9·03
0·085	2·292	15·20	0·053	1·444	0·1596	8·38
0·064	1·992	15·50	0·040	1·316	0·1192	7·89
0·048	1.789	16·44	0·030	1·224	0·0878	7·47
0·036	1·641	17·81	0·0225	1·161	0·0648	7·16
0·027	1·502	18·59	0·0180	1·124	0·0508	6·89
0·0203	1·403	19·85	0·0135	1·092	0·0382	6·81
0·0152	1·308	20·26	0·0101	1·066	0·0277	6·53
0·0114	1·249	21·84	0·0076	1·050	0·0212	6·58
Zero	1·000	—	Zero	1·000	0·0000	(6·45)

TABLE 5. — COMMERCIAL APPLE PECTIN, VISCOSITY AT 20° C.

Medium:	H_2O		0·2 N-NaCl		
Conc. wt. per cent	η_r	η_{sp}/c	η_r	log η_r	η_{sp}/c
1·0	17·85	16·85	23·55	—	—
0·8	11·38	12·98	13·95	—	—
0·6	6·94	9·90	7·78	—	—
0·4	4·10	7·75	4·45	—	—
0·206	2·315	6·38	2·209	0·3441	5·87
0·154	1·970	6·30	1·809	0·2574	5·25
0·116	1·759	6·54	1·557	0·1923	4·80
0·087	1·581	6·68	1·409	0·1488	4·70
0·065	1·485	7·46	1·280	0·1072	4·31
0·0325	1·301	9·26	1·129	0·0526	3·97
0·0163	1·186	11·41	1·062	0·0261	3·80
Zero	1·000	—	1·000	0·0000	(3·84)

Table 6. — Beet Pectin, Viscosity at 20° C.

Medium:	H_2O		0·2 N-NaCl			
Conc. wt. per cent	η_r	η_{sp}/c	Conc. wt. per cent	η_r	log η_r	η_{sp}/c
0·400	2·926	4·82	0·200	1·798	0·2548	3·99
0·300	2·409	4·70	0·133	1·484	0·1714	3·64
0·225	2·038	4·61	0·089	1·310	0·1173	3·48
0·169	1·795	4·70	0·059	1·196	0·0778	3·32
0·127	1·643	5·06	0·040	1·128	0·0523	3·20
0·095	1·514	5·41	0·026	1·081	0·0338	3·12
0·071	1·408	5·75	Zero	1·000	0·0000	(2·93)
0·053	1·348	6·57				
0·040	1·269	6·73				
0·030	1·211	7·03				
0·0225	1·169	7·51				
0·0113	1·094	8·32				
Zero	1·000	—				

Table 7. — Alginic Acid, Viscosity at 20° C.

Medium:	0·2 N-Na_2CO_3		
Conc. wt. per cent	η_r	log η_r	η_{sp}/c
0·415	7·648	0·8836	16·02
0·208	3·272	0·5148	10·92
0·104	1·907	0·2804	8·72
0·052	1·451	0·1617	8·67
0·026	1·222	0·0871	8·54
Zero	1·000	—	(7·72)

The quotient η_{sp}/c is of a special interest owing to its significance in the well-known molecular weight relation of Staudinger (58). As may be seen from Tables 4—8 and Fig. 6, the value of η_{sp}/c for the substances in pure water passes through a minimum — except for gum arabic — and increases strongly as concentration approaches zero. This course, which deviates markedly from that obtained with

Table 8. — Gum Arabic, Viscosity at 20° C.

Medium:	H_2O		0·2 N-NaCl			
Conc. wt. per cent	η_r	η_{sp}/c	Conc. wt. per cent	η_r	log η_r	η_{sp}/c
3·18	2·416	0·45	2·84	1·695	0·2292	0·24
1·59	1·811	0·51	1·60	1·346	0·1290	0·22
0·80	1·510	0·64	1·20	1·250	0·0969	0·21
0·40	1·323	0·81	0·80	1·162	0·0653	0·20
0·20	1·211	1·06	0·40	1·080	0·0334	0·20
0·10	1·129	1·29	0·30	1·061	0·0257	0·20
0·05	1·072	1·44	0·20	1·039	0·0166	0·20
0·025	1·037	1·48	0·10	1·019	0·0082	0·19
0·0125	1·019	1·52	0·05	1·011	0·0048	0·22
Zero	1·000	—	Zero	1·000	—	(0·20)

non-polar substances, is most striking in the case of citrus pectin and becomes progressively less pronounced for apple pectin, beet pectin and gum arabic. A similar course for the η_{sp}/c, c-relation was demonstrated by Kruyt and co-workers (59) for agar and gum arabic and by Kern (60) for polyacrylic acids in aqueous solutions. In the latter case substances of varying degrees of polymerization, containing from 50 to 1 000 carboxyl groups per particle, were investigated. It was found that the η_{sp}/c minimum was more distinctly developed the higher the degree of polymerization, and, by analogy, citrus pectin might be expected to have the highest degree of polymerization among the pectins. Gum arabic must be left out of the comparison in this respect owing to its different structure.

The anomalous viscosity properties of the polybasic acids are obviously due to the charges of the dissociated carboxyl groups. Purified methyl cellulose, for instance, did not show any deviations of this kind. According to Kern (*l. c.*), the anomalous behaviour may be interpreted formally by assuming the co-operation of two factors, viz. the 'ionic factor', I, and the 'macromolecular factor', $(\eta_{sp}/c)_m$, allowing for the influence of the charges and the degree of polymerization respectively upon viscosity, thus

$$\eta_{sp}/c = I \cdot (\eta_{sp}/c)_m. \qquad (1)$$

The influence of the ionic factor depends upon the degree of dissociation of the acid and asserts itself only at lower concentrations, where the macromolecular factor is assumed to vary only slightly. The latter factor depends upon the length of the molecules, and determines the magnitude of the viscosity at higher concentrations.

The molecular mecanism behind the phenomena is not, however, discussed by Kern. According to Pauli and Valkó (61) the degree of hydration of the particles will increase as concentration decreases, owing to the fact that the distance between the particles and the compensating ions ('Gegenionen') grows larger and thus allows the water dipoles to approach closer to the particles. The effective volume of the particles will thus increase. In addition, we might assume, as suggested by Born (62) in the case of ionic migration, that the orientation of the water dipoles is disturbed when the particles move, and as a consequence a continuous rearrangement will take place, manifesting itself as a viscous drag. An increase in concentration, or the addition of a foreign electrolyte, on the other hand, causes a denser ionic atmosphere around the particles and thus a reduction in hydration and viscosity.

Kruyt and co-workers (59) also stress the rôle played by hydration. Much of their reasoning is based, however, on the so called 'electroviscous effect', which was first expressed mathematically by Smoluchowski (63) on the basis of the well-known Einstein viscosity equation, and later deduced by Krasny-Ergen (64),

$$\eta_r - 1 = 2{\cdot}5\,\varphi\left[1 + k\frac{1}{\varkappa a^2}\frac{1}{\eta_0}\left(\frac{\varepsilon\zeta}{2\pi}\right)^2\right] \qquad (2)$$

where φ is the volume of the particles relative to the volume of the solution, $\varkappa$ the specific conductivity of the solution, a the radius of the particles, η_0 the viscosity and ε the dielectric constant of the solvent, and ζ the electrokinetic potential of the particles. The value of k was 1 in Smoluchowski's deduction and 3/2 in that of Krasny-Ergen.

It was emphasized by Smoluchowski, however, that this equation was in fact valid only for suspended particles approximately spherical in shape, its applicability to hydrophilic colloids, especially those of high conductance, is doubtful.

Owing to the uncertainty of the values of φ and a, Kruyt does not use Eq. (2) for numerical calculations. He shows, however, that

η_r is reduced on addition of an electrolyte, and attributes this fact mainly to the increase in conductivity, which, according to Eq. (2) will bring about a decrease in the electroviscous effect. Kruyt thus looks upon the charge effects as being electrocapillary phenomena, while Pauli and Valkó consider them to be of an electrolytic nature. The latter conception seems to be the more natural one in the case of the polyuronides. On the other hand, the equivalence of the diffuse double layer and Debye-Hückel's 'ionic atmosphere' has made the distinction between the two contrasting conceptions a rather illusive one (cf., for instance, Hartley, 65).

It is interesting to note that the characteristic course of the $(\eta_{sp}/c, c)$-curves for the polyuronides in pure water appears almost equally marked in the case of several simple electrolytes, e. g. $MgSO_4$, $LiIO_3$, $LiNO_3$, $CsNO_3$, $BaCl_2$, $LaCl_3$, $K_4Fe(CN)_6$ etc.

From measurements on $BaCl_2$ solutions, Jones and Dole (66) arrived at an expression for the way in which the relative fluidity $\varphi = 1/\eta_r$ depends upon concentration c, thus

$$\varphi = 1 - A\sqrt{c} + Bc \tag{3}$$

which, for low concentrations, is equivalent to

$$\eta_r = 1 + A\sqrt{c} + B'c, \tag{4}$$

or

$$\eta_{sp}/c = \frac{A}{\sqrt{c}} + B', \tag{5}$$

where A and B' are constants. As may be seen from this equation, η_{sp}/c approaches infinity as c decreases.

The theoretical foundation of Eq. (4) was given by Falkenhagen (67), who was able to show that the expression

$$\eta_r = 1 + A\sqrt{c} \tag{6}$$

may be obtained by taking into account the deforming effect that the velocity gradient in the capillary flow exerts upon the ionic atmosphere surrounding the ions. The constant A was defined by a mathematical expression including the valency and mobility factors for the ions, the absolute temperature, and the viscosity and dielectric constant of the medium. The value of A was found to be positive

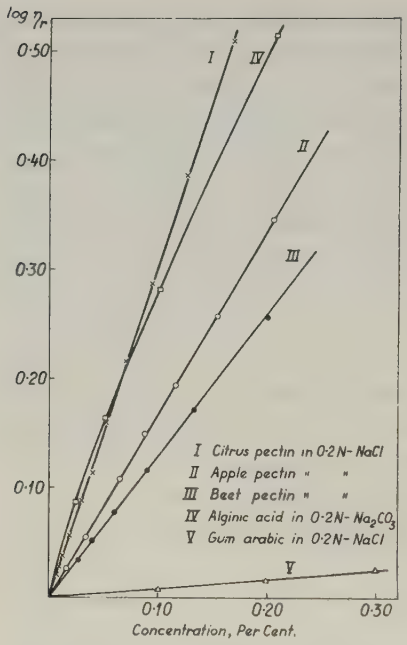

Fig. 7.—log η_r for the polyuronide type substances in electrolytic media, as a function of concentration.

for all kinds of electrolytes. — So far, it has not been possible to deduce a corresponding expression for B' in Eq. (4).

In any case it seems very reasonable to assume that effects of the kind described by Falkenhagen in the case of simple electrolytes are partly responsible for the viscosity anomalies of the polyuronide solutions too (cf. Philippoff, 68).

When a salt is added to the solutions of the polyuronides, the viscosity anomalies due to the charges disappear or are greatly reduced. This may be seen from Tables 4—8. In fig. 7 the values of log η_r (Briggsian logarithms) from measurements in 0·2 N salt solutions are plotted against concentration. Except for alginic acid, the relation is strictly linear at least up to a concentration of about 0·3 per cent, thus conforming to the Arrhenius viscosity equation for electrolytes (69). Owing to this fact it is easy to obtain the 'intrinsic viscosity', $[\eta]$, (Table 9), i. e. the characteristic viscosity constant defined by Kraemer and Lansing (70),

$$[\eta] = \left(\frac{\ln \eta_r}{c}\right)_{c\to 0} = (\eta_{sp}/c)_{c\to 0}. \qquad (7)$$

According to Staudinger (58, p. 64) this quantity is proportional to the molecular weight, even in the case of heteropolar substances, provided the molecules have the proper chain structure and the measure-

Table 9. — Intrinsic Viscosity of the Type Substances.

Citrus pectin	Apple pectin	Beet pectin	Alginic acid	Gum arabic
6·45	3·84	2·93	7·72	0·20

ments are performed on solutions containing excess of electrolyte. Whether there really is any simple relation between the $[\eta]$-values and the molecular weight of pectins and alginic acid will be discussed later on (p. 94).

The viscosity of pectin solutions is of special interest in so far as it has been assumed to be an indication of the jellying power of the substances. Hardy (71), for instance, stated that 'viscosity is a measure of the degree of incipient gelation'. Recent investigations made by Ripa (48, p. 160), Meyers and Baker (49), Bennison and Norris (72), Olsen and co-workers (55) and others have shown, however, that viscosity data are not very reliable in this respect. There seems to be a certain relationship between viscosity and jellying power only where the substances are derived from the same source and by the same method of preparation. For a given substance, however, viscosity and jellying power seem to be affected by various factors in a similar way.

As will be shown in the next section, the hydrogen ions play a fundamental rôle in the jellying process; their influence on viscosity will therefore be demonstrated here.

In Fig. 8 η_r is plotted against the pH of the solution. The pectin concentration is of the order of magnitude 1 per cent in all cases, i. e. a concentration commonly used in jelly making. pH was varied by addition of hydrochloric acid or sodium hydroxide to the test solution. For the purpose of comparison some measurements on solutions of methyl cellulose were also recorded.

Apple pectin (Fig. 8 *A*, I). A stock solution was neutralized with sodium hydroxide and test solutions were prepared by adding increasing amounts of hydrochloric acid and the proper amount of water to weighed amounts of the stock solution.

The curve exhibits a very distinct maximum at pH 2·9, a minimum at pH about 3·7, and a second maximum in the neighbourhood of pH 6. This peculiar course of the curve is probably due to the regulating effect of the carboxyl groups upon degree of hydration and magnitude of the intermolecular forces. When HCl is added the degree of dissociation of the carboxyl groups decreases and, probably in consequence, the degree of hydration, and hence the viscosity. This will proceed to a minimum beyond which the viscosity, will within a rather narrow pH range, depend strongly upon the action of cohesive forces among the particles. At pH 2·9 these forces have

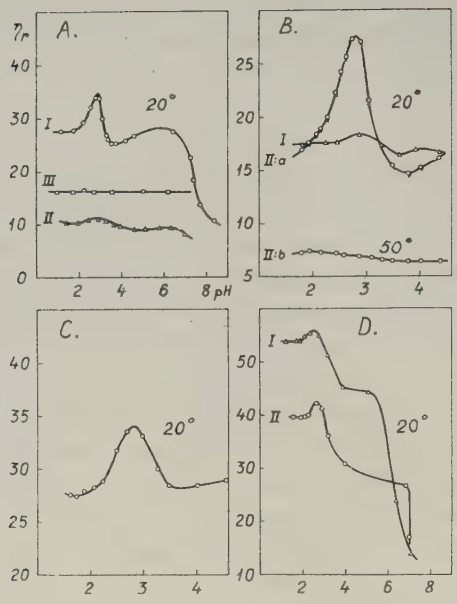

Fig. 8.—Variation of the relative viscosity with pH. (Cf. text.)

A, I. Commercial apple pectin: stock solution neutralized with NaOH, concentration 1·3 per cent. No sodium chloride added. II. Beet pectin in 0·2 N-NaCl, concentration 1·0 per cent. III. Methyl cellulose in 0·2 N-NaCl, concentration 1·0 per cent.

B. Commercial apple pectin: stock solution acidic, concentration 1·0 per cent, initial pH = 2·55. I. Viscosity of salt-free solutions. II. Viscosity of solutions containing NaCl (0·2 N), a) referring to measurements at 20° C, b) referring to measurements at 50° C.

C. Commercial apple pectin in a 25 per cent solution of sucrose, concentration 1·0 per cent.

D. Citrus pectin: stock solution acidic, concentration 1·0 per cent, initial pH = 2·78. I. Viscosity of salt-free solutions. II. Viscosity of solutions containing NaCl (0·2 N).

a maximum, thus it would perhaps be justified to talk about 'incipient gelation' at this point. In the neighbourhood of this pH the jellying power also has a maximum, as will be shown in the next section.

The presence of a certain amount of salt is necessary to obtain a clear development of the viscosity maximum. This is clearly shown in Fig. 8, *B*. Curve I gives the viscosity of solutions where the pH was varied with the necessary amounts of HCl and NaOH, starting from a stock solution with a pH of its own equal to 2·55. Curve II refers to solutions containing in addition NaCl to a concentration of 0·2 N. The viscosity of the latter solutions was measured first at 20° C (II: a, ⊙), then at 50° C (II: b) and finally at 20° C again

(II: a, ×). As may be seen the maximum disappears at 50° C but reappears at 20° C, the values of the viscosity obtained in the first and the third measurement being practically identical. The thermal agitation at 50° C is thus strong enough to outweigh the cohesive forces.

In the case illustrated in Fig. 8 *A*, I, a sufficient amount of salt was secured for the development of the maximum by the neutralization and the subsequent addition of HCl.

Sodium chloride may be exchanged for sucrose, as might be inferred from fig. 8 *C*, considerably larger amounts of the latter substance being required, however, for the attainment of equally marked maxima.

Beet pectin (Fig. 8 *A*, II). The variation of viscosity with pH is very small even in the presence of sodium chloride. There is only an indication of a maximum in the diagram. The jellying power of this substance was also practically nil. The reason why this pectin behaves differently from apple pectin is not clear. The presence of acetyl groups in the molecule (p. 21) is an indication, however, that peculiarities in constitution may be responsible.

Methyl cellulose (Fig. 8 *A*, III). — Solutions of methyl cellulose in 0·2 N-NaCl, having a viscosity comparable to that of apple pectin outside the maximum range, exhibited no variations in viscosity as pH was varied. This fact may be attributed to the absence of carboxyl groups in the molecule.

Citrus pectin (Fig. 8 *D*). The viscosity has a maximum value in the neighbourhood of pH 3, whether there is any sodium chloride present or not. The addition of sodium chloride causes a general decrease in viscosity, but has no particularly marked influence upon the viscosity in the maximum range.

The difference in the influence of sodium chloride upon the viscosity of the apple pectin and the citrus pectin solutions is certainly partly due to the difference in the number of free carboxyl groups in the molecule, and may perhaps, be also due to the difference in the size of the molecules (cf. Table 36).

The variation of viscosity with pH described here was equally marked if some other strong acid was substituted for hydrochloric acid. With decreasing concentration of pectin the characteristic maximum gradually decreased, but, in the case of apple pectin, a tendency to increasing viscosity could still be observed at pH 3 when the concentration was as low as 0·2 per cent.

THE JELLYING PROPERTIES OF THE TYPE PECTINS.

One of the most important and widely investigated properties of pectin is its capacity to form rigid jellies with the appropriate quantities of sugar or glycerine and acid. It may not be out of place, therefore, to make an attempt to differentiate between the type pectins dealt with here as regards this property. It will be of particular interest to see later to what extent the jellying power and the molecular weight of the substances are connected.

The apparatus shown in Fig. 9 was devised for measuring the strength of the pectin jellies.[1] The measurements by means of this

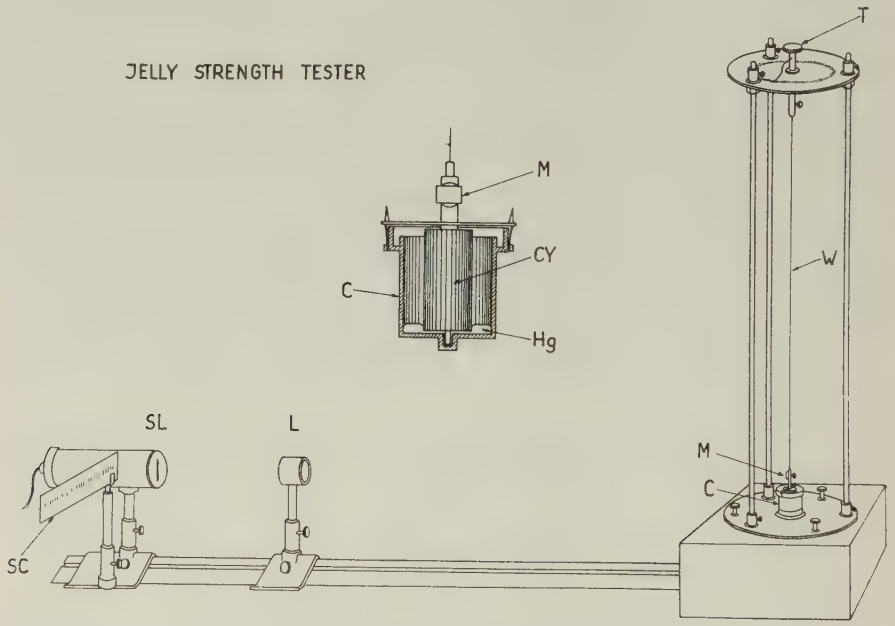

Fig. 9.—Apparatus for measuring jelly rigidity.

C is the jelly holder, *CY* cylinder for applying a shearing stress to the jelly, *Hg* mercury cushion, *M* mirror, *T* torsion head, *W* torsion wire, *SL* illumination slit, *L* lens and *SC* scale.

[1] The apparatus may now be obtained from LKB-Produkter, Fabriksaktiebolag, Stockholm.

Fig. 10.—Pectin jelly containing 70 per cent glycerine and 1 per cent commercial apple pectin.

apparatus are based on the establishment of an equilibrium between the forces developed in the jelly and those set up in a torsion wire when it is being twisted.

CY is a cylinder suspended by the torsion wire *W* within the jelly holder *C* and held in position by a steering device. The holder and cylinder are made of stainless steel, and grooved as indicated in Fig. 9 in order to prevent slipping between the jelly and the metal surfaces when measurements are being made. The bottom of the holder is covered with mercury in order to eliminate bottom effects.

The torsion of the wire *W* is determined by means of the scales at *T* and *SC*, Fig. 9, as will be described below.

Pectin jellies were prepared in the following way. Calculated amounts of a pectin solution, glycerine or a sugar solution, water and dilute hydrochloric acid were mixed in a beaker to a total weight of 30 grams. The mixture was stirred intensively and heated on an asbestos wire gauze until it boiled, i. e. for about 4 to 5 minutes. The beaker was then removed and a quantity of water equal to that lost in heating was stirred in. The mixture was then poured into the cup *C* of the jelly strength tester, Fig. 9, and allowed to stand for twenty-four hours. The jelly thus obtained assumes the shape of a cylindrical ring as shown in Fig. 10.

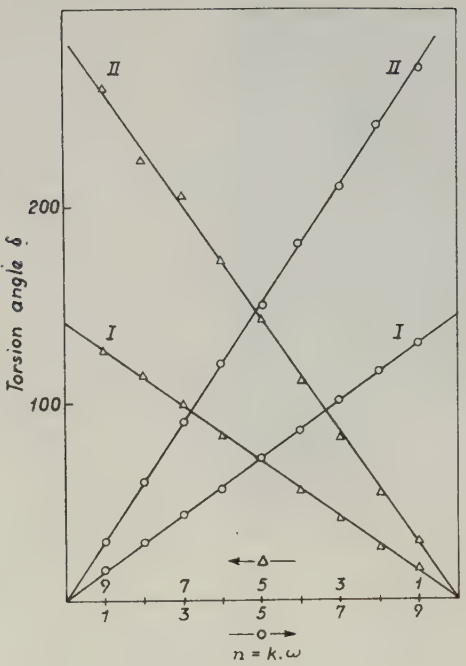

Fig. 11.—Relation between the torsion angle δ of the wire W, Fig. 9, and the shearing angle ω of an apple pectin jelly (I), and a citrus pectin jelly (II) of equal composition.

The rigidity of the jelly, as measured by means of the jelly strength tester, was taken as characteristic of the jellying property of the pectin.

On turning the torsion head T, Fig. 9, through an angle θ, a shearing stress is applied to the jelly ring by means of the torsion wire W and the cylinder CY, equilibrium being attained when the cylinder has turned through an angle ω. Owing to the fact that the jelly is resting on a layer of mercury the bottom of the cup exerts no deforming stress upon it. The rather small angle ω is determined by aid of a light beam reflected by the mirror M on to the scale SC. The shear modulus or rigidity G of the jelly may be calculated from the equation

$$G = \frac{M}{4\pi h}\left(\frac{1}{r^2} - \frac{1}{R^2}\right)\frac{\delta}{\omega} \qquad (8)$$

deduced by Schwedoff (73).

M is the torsion modulus of the given wire, h the height, r the inner and R the outer radius of the jelly cylinder. $\delta = \theta - \omega$ is the torsion angle of the wire. G is expressed in dynes/cm^2.

The numerical values of the constant factors were the following. $h \approx 3{\cdot}3$ cm, $r = 1{\cdot}00$ cm, $R = 1{\cdot}75$ cm and $M = 10\,200$ dynes.cm. The latter value refers to a torsion wire made of steel ('piano wire'), 54·8 cm long and 0·051 cm in diameter. M was calculated from data obtained in an oscillating experiment, the wire being fixed to the axis of a cylinder of known moment of inertia.

On substituting the values of the constants Eq. (8) is transformed into

$$G = 166 \cdot \frac{\delta}{\omega} \text{ dynes/cm}^2. \qquad (9)$$

TABLE 10. — DEPENDENCE OF THE JELLY RIGIDITY UPON PECTIN CONCENTRATION.

Glycerine Conc.: 70 per cent. Acidity: 6 Ml 0·1 N-HCl per 100 g of the Jelly.

Percentage pectin	$G \cdot 10^{-3}$	
	Citrus pectin	Apple pectin
0·50	6·6	2·9
0·75	18·5	8·7
1·00	42·7	16·8
1·46	—	33·9

TABLE 11. — DEPENDENCE OF THE JELLY RIGIDITY UPON GLYCERINE CONCENTRATION.

Pectin Conc.: 1 per cent. Acidity: 6 Ml 0·1 N-HCl per 100 g of the Jelly.

Percentage glycerine	$G \cdot 10^{-3}$	
	Citrus pectin	Apple pectin
33	1·4	—
40	13·5	—
50	47·6	1·1
60	50·1	10·4
70	42·7	20·3
88	—	21·6

TABLE 12. — DEPENDENCE OF THE JELLY RIGIDITY UPON ACIDITY.

Pectin Concentration: 1 per cent.

Ml 0·1 N-HCl per 100 g of the jelly	Citrus pectin jellies containing				Apple pectin jellies containing			
	glycerine, 50 per cent		sugar, 50 per cent		glycerine, 70 per cent		sugar, 50 per cent	
	pH	$G \cdot 10^{-3}$	pH	$G \cdot 10^{-3}$	pH	$G \cdot 10^{-3}$	pH	$G \cdot 10^{-1}$
0·0	3·57	2·6	3·25	20·3	2·77	—	2·45	3·6
1·8	—	—	—	—	—	—	2·34	9·2
3·0	3·30	29·0	2·68	39·0	2·77	9·4	2·51	15·4
6·0	2·85	37·1	—	—	2·38	16·8	2·09	19·7
9·0	2·62	40·2	—	—	—	—	1·90	15·4
12·0	2·13	42·1	—	—	2·32	14·7	1·98	15·4
18·0	2·73	13·5	—	—	1·83	12·3	—	—
24·0	—	—	—	—	1·82	6·2	—	—

Fig. 11 gives an example of the relation between δ and $n = k \cdot \omega$, where n is the displacement in cm of the slit image on the scale (cf. Fig. 9), and k has the value 3·74 cm/degree. The measurements were not usually extended beyond $n = 10$, corresponding to a value of ω equal to about 2·7°.

As may be seen δ varies linearly with n. For values of ω up to

Table 13. — Dependence of the Rigidity of Apple Pectin Jellies upon Concentration of Hexamine Cobaltichloride.

Pectin Concentration: 1 per cent. Glycerine Concentration: 70 per cent.

Number of equivalents ($\times 10^5$) of $Co(NH_3)_6Cl_3$ per 100 g of the jelly	Number of equivalents of $Co(NH_3)_6Cl_3$ per equivalent of pectin	pH	$G \cdot 10^{-3}$
10	0·04	2·67	0·14
33	0·14	2·70	2·9
67	0·29	3·06	16·1
133	0·58	3·66	52·0

about 1° ($n = 3{\cdot}74$) the jellies obtained with apple and citrus pectin usually behaved as perfectly elastic bodies. For higher values of ω (2 à 3°) relaxation phenomena appeared.

In Tables 10—13 and Fig. 12 the variation of the jelly rigidity with the composition of the jelly is illustrated. The data refer to citrus and apple pectin jellies. Any attempt to make jellies with beet pectin failed, a common experience among workers on pectin (cf., for instance, Bennison and Norris, 72). Flax pectin also gave very weak jellies.

The jellying power of the citrus pectin is obviously far greater than that of the apple pectin (Fig. 12 *A*). The rigidity of the apple pectin jellies exhibits a maximum at a pH of about 2·4 under the conditions stated in Table 12 and Fig. 12 *B*. This fact is probably due to the action of forces of the kind which were manifest in the viscosity measurements (p. 31).

The rigidity of the citrus pectin jellies increases with increasing acidity to about pH 2. On further addition of acid there is a sudden decrease in rigidity to a fraction of the highest value. This decrease is certainly due to a decomposition, perhaps in the first place a demethoxylation, brought about by the hot acid. The simultanous rise of pH from 2·1 to 2·7 may probably be attributed to an increase in the buffering capacity of the pectin, due to an increase in the number of free carboxyl groups by the demethoxylation.

The extent to which the jelly rigidity depends upon glycerine concentration (Fig. 12 *C*) also exhibits a maximum value. This fact seems to be simply a saturation phenomenon. On preparing citrus pectin

jellies containing 70 per cent glycerine and 1 per cent pectin, for instance, it was repeatedly observed that small flocks of pectin remained undissolved, even on prolonged boiling. Incomplete dissolution brought about less rigid jellies. Apple pectin dissolved to a concentration of 1 per cent even at a glycerine concentration of 88 per cent.

In table 12 some data are also given for jellies prepared with sugar instead of glycerine. The superiority of the citrus pectin in forming jellies manifests itself again.

It will be clear from the figures collected in Table 13 that other factors in addition to those considered above, may influence the jelly formation. They refer to jellies prepared from apple pectin, glycerine and varying amounts of $Co(NH_3)_6Cl_3$[1], while no acid was added. As may be seen the rigidity of the jellies strongly increases with the amount of hexamine cobaltichloride, attaining values considerably higher than those obtained with acid instead of salt (Table 12).

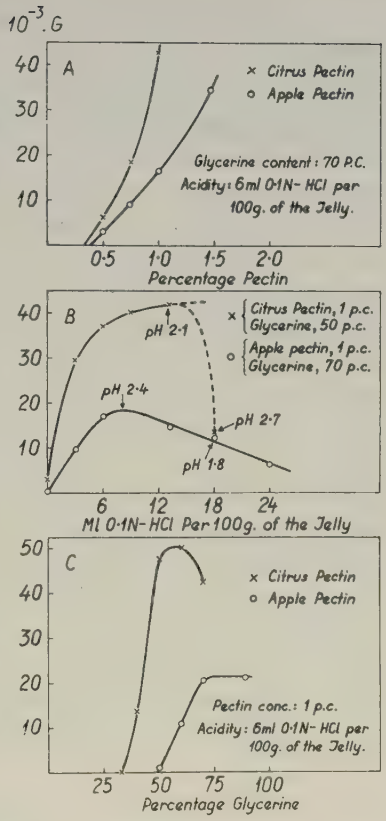

Fig. 12.—Variation of the jelly rigidity with composition of the jelly.

The mecanism active in forming these kinds of jellies undoubtedly involves some new factor, probably the formation of 'bridges' between the carboxyl groups of adjacent particles by means of the trivalent cations, in just the same manner as suggested by Henglein in the case of protopectin (p. 12). As a matter of fact, jellies of this rigidity could not be obtained by means of univalent cations of any concentration.

It should be mentioned that the jellies containing hexamine cobaltichloride, particularly those of the highest rigidity (Table 13),

[1] This salt was found most suitable for easily obtaining desired amounts of trivalent cations. It is not hydrolyzed to any large extent, is very resistant to heat and does not generally bring about flocculation of the pectin.

were found to be much more brittle than those prepared by means of an acid.

The factors determining the jellying power of the pectins have been widely discussed. The percentage methoxyl was considered for some time to be of fundamental importance. According to Myers and Baker (20), however, 'no single constituent of the pectin molecule is a criterion of its jellying power'. This power is attributed by them to the degree of polymerization of the poly-galacturonic acid. Schneider and Bock (74) are of the same opinion. Hinton (75), on the other hand, divides the factors into two groups, '(a) the inherent quality of the pectin, which varies with the source and the degree of modification that may have been effected in extraction; and (b) circumstances in the actual jellying process, such as pH conditions and the presence of salts.

The magnitude of the jellying power governed by the first group of factors is probably dependent in some way on the molecular size and form of the pectin molecule, whereas the degree to which this power is manifest in actual jellies depends on the properties of the non-esterified carboxyl groups in the molecule and their relation with anion and cation constituents of the jellying mixture' (Norman, 76).

We shall return to the problem when discussing the molecular weight of the pectins dealt with here.

SOME ELECTROCHEMICAL PROPERTIES.

The acid polyuronides are colloidal electrolytes. They may be given the common formula $P \cdot (COOH)_n$ and their dissociation in aqueous solution outlined as follows.

$$P \cdot (COOH)_n \rightleftarrows P \begin{cases} (COO^-)_x \\ (COOH)_{n-x} \end{cases} + x\,H^+. \qquad (10)$$

As was shown above the electrolytic properties of the substances manifest themselves strongly in the measurements of viscosity and jellying power. In the subsequent chapters it will be shown that the sedimentation and diffussion of the substances are still more pronouncedly influenced by the charges. Because of this, the present chapter will be devoted to the characterization of the polyuronides from a purely electrochemical point of view.

CONDUCTIVITY.

The data in Tables 14—19 refer to two of the type pectins and to gum arabic. The solutions investigated were purified by electrodialysis for one or two days. The conductivity measurements were performed with the aid of a cell having the constant 0·1583 cm^{-1}, determined in the manner described by Jones and Bradshaw (77). The water used for dilution had a specific conductivity equal to about $2 \cdot 10^{-6}$ $ohm^{-1} \cdot cm^{-1}$. The pH values were measured with a glass electrode and a vacuum tube potentiometer. All measurements were made at 20° C.

A. Acidic Substances. — The conductivity of the solutions of the acidic polyuronides seems to be due to the hydrogen ions alone. At the lowest concentrations the measured conductivity $\varkappa$ is practically equal to the value $\varkappa_H$, calculated from the hydrogen ion activity on the assumption that the mobility of the hydrogen ion is equal to that

TABLE 14.[1] — ELECTRODIALYSED APPLE PECTIN, EQUIV. WEIGHT 435. — TEMP.: 20° C.

Concentration per cent	$c_E \cdot 10^4$	pH	$c_H \cdot 10^4$	$\varkappa \cdot 10^4$	$\varkappa_H \cdot 10^4$	Λ	Λ_H	Degree of diss. per cent	$k \cdot 10^4$	η_r
1·203	276·6	2·55	28·2	9·15	9·17	33	324	10	3·2	27·06
0·602	138·4	2·72	19·1	6·16	6·21	45	323	14	3·1	6·95
0·301	69·2	2·90	12·6	4·04	4·10	58	321	18	2·8	3·10
0·150	34·5	3·07	8·51	2·57	2·77	74	302	25	2·8	1·93
0·075	17·2	3·30	5·01	1·55	1·63	90	309	29	2·1	1·52
0·0375	8·62	3·50	3·16	0·92	1·03	106	291	37	1·8	1·38
0·0188	4·32	3·78	1·66	0·52	0·54	120	313	38	1·0	1·24
0·0094	2·16	4·05	0·89	0·28	0·29	130	315	41	0·62	1·18

TABLE 15.[2] — ELECTRODIALYSED CITRUS PECTIN, EQUIV. WEIGHT 677. — TEMP.: 20° C.

Concentration per cent	$c_E \cdot 10^4$	pH	$c_H \cdot 10^4$	$\varkappa \cdot 10^4$	$\varkappa_H \cdot 10^4$	Λ	Λ_H	Degree of diss. per cent	$k \cdot 10^4$	η_r
0·50	73·9	2·70	20·0	5·98	6·25	81	299	27	7·4	15·91
0·25	36·9	2·87	13·5	3·73	4·39	101	276	37	7·8	4·82
0·125	18·5	3·12	7·59	2·21	2·47	119	291	41	5·3	2·49
0·063	9·30	3·34	4·59	1·39	1·49	151	303	49	4·5	1·83
0·031	4·57	3·59	2·58	0·77	0·84	167	298	56	3·3	1·48
0·016	2·36	3·91	1·25	0·42	0·41	183	336	52	1·4	1·29
0.008	1·18	4·20	0·63	0·21	0·21	175	333	53	0·7	1·20

at infinite dilution. As concentration increases $\varkappa$ becomes smaller than $\varkappa_H$, no doubt as a result of a decrease in the mobility of the hydrogen

[1] c_E is the number of pectin equivalents per litre of solution, c_H hydrogen ion concentration in equivalents per litre, $\varkappa$ the measured specific conductivity of the solution, $\varkappa_H$ the specific conductivity of the hydrogen ions as calculated on the assumption that the ionic equivalent conductivity is equal to that at infinite dilution, i. e. 325 ohm$^{-1} \cdot$ cm^2 at 20° C, Λ the equivalent conductivity of the solution, Λ_H the equivalent conductivity on the basis of the ionized fraction of the substance, and k the apparent dissociation 'constant'.

[2] The symbols are those used and explained in Table 14.

TABLE 16.[1] — ELECTRODIALYSED GUM ARABIC, EQUIV. WEIGHT 1200. — TEMP.: 20° C.

Concentration per cent	Concentration $c_E \cdot 10^4$	pH	$c_H \cdot 10^4$	$\varkappa \cdot 10^4$	$\varkappa_H \cdot 10^4$	Λ	Λ_H	Degree of diss. per cent	$k \cdot 10^4$	η_r
3·16	263 3	2·44	36·3	9·91	11·80	38	273	14	5·8	2·42
1·58	131·7	2·67	21·4	5·85	6·95	44	273	16	4·2	1·83
0·79	65·8	2·90	12·6	3·29	4·09	50	261	19	3·0	1·51
0·40	33·3	3·16	6·92	1·77	2·25	53	256	21	1·8	1·31
0·20	16·7	3·46	3·47	0·96	1·13	57	277	21	0·9	1·19
0·10	8·33	3·75	1·78	0·50	0·58	60	281	21	0·5	1·12
0·05	4·16	4·10	0·79	0·25	0·26	60	316	19	0·2	1·06

ion. The discrepancy between the two values increases with increasing equivalent weight.

In any case the transference number of the colloidal ion, if it differs from zero, must be very low in these solutions. Similar results were obtained by Pauli and Ripper (78) and by Briggs (79) from investigations of gum arabic, and by Kern (60) in his study of the polyacrylic acids quoted above (p. 27). According to Kern the high molecular chain-shaped anions form a network that does not take any measurable part in the current transport and which, while strongly contributing to the macro viscosity, offers no resistance to the migration of the hydrogen ions. — The viscosity figures given in Tables 14—16 confirm, it seems, the non-importance of the macro viscosity as regards the conductivity of the hydrogen ions.

Values of the equivalent conductivity Λ, calculated in the usual manner, and of Λ_H calculated on the basis of the ionized fraction of the substance, are recorded in Tables 14—16, mainly for the purpose of comparison with the results obtained by Briggs (*l. c.*) from his measurements on gum arabic. In Fig. 13 Λ_H is plotted against (total) equivalent concentration. As may be seen, the curves for the pectins, like that for gum arabic, exhibit a minimum at a concentration of about $40 \cdot 10^{-4}$ equivalents per litre in all cases. Briggs also found a minimum in the curve for gum arabic at a concentration of about $25 \cdot 10^{-4}$ equivalents per litre, and discusses the possible reasons for it.

[1] The symbols are those used and explained in Table 14.

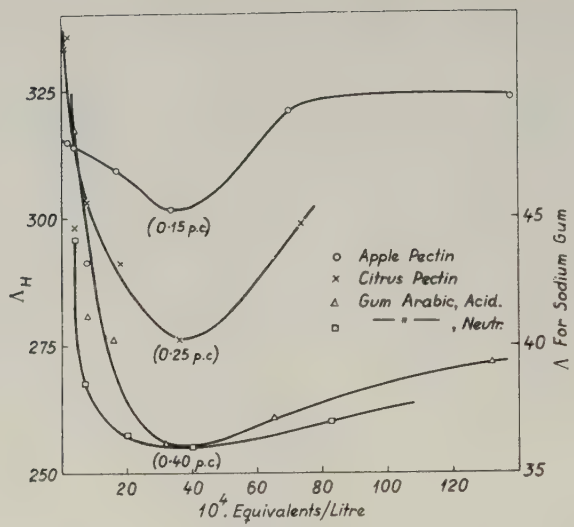

Fig. 13.—Variation of the equivalent conductivity with equivalent concentration. Λ_H means equivalent conductivity on the basis of the ionized fraction of the acids.

(a) The increase in conductivity at the lowest concentrations may be due, he assumes, to increasing ionization, while at concentrations higher than that at which the minimum is observed the conductivity may increase owing to an increase in the degree of aggregation of the colloidal anion accompanied by a marked increase in the charge density and hence in the mobility of the micella.

Anticipating the results obtained from sedimentation and diffusion measurements, it may be stated here that no evidence has been found supporting the conception of a varying degree of aggregation within this concentration range.

(b) From surface conductance measurements on glass and cellulose surfaces, Briggs (80) concludes that 'the ions in the region of the interface can move more freely through the surface region under the influence of an electric field than they can through the bulk of the solution'. It may therefore be possible that, since an increase in concentration causes a greater number of contacts between the particles, there will be a marked increase in the current carried by surface conducting ions when the concentration of the minimum is passed.

A network of the kind suggested by Kern would, of course, offer particularly suitable conditions for surface conductance.

(c) Finally it is possible that the mobility of the compensating ions may vary with the concentration in such a way as to give rise to the minimum. Briggs refers to Lewis, who discovered the existence of a minimum in the transference number-concentration curve for several ions in true solution.

B. Neutralized Substances. — If sodium ions are substituted for the hydrogen ions by neutralizing with sodium hydroxide, the conductivity of the pectins resembles more closely that of normal low molecular electrolytes. The behaviour of gum arabic is, however, still anomalous.

From the conductivity data in Tables 17—19 it may be seen that the pectin anions now take a measurable part in the current transport and that values for their mobility u_k may be calculated. The accuracy of the values may, of course, be impaired by a rather large error, especially at higher concentrations, as we know little about the real value of the mobility of the sodium ion and of the degree of ionization at these concentrations. It will be shown later, however, that the magnitude of u_k at the lowest concentrations agrees rather well with that determined electrophoretically.

The increase in the mobility of the pectin anions on neutralization is probably due to the co-operation of various factors. Salt formation

Table 17.[1] — Neutralized Apple Pectin. — Temp.: 20° C.

Concentration		pH	$\varkappa \cdot 10^4$	$\varkappa_{Na} \cdot 10^4$	$u_k \cdot 10^5$	Λ_{Na}	Λ
per cent	$c_E \cdot 10^4$						
0·500	118	7·17	6·21	4·96	11	42	53
0·250	59	6·94	3·33	2·54	14	43	56
0·125	29·50	6·73	1·78	1·30	17	44	60
0·063	14·75	6·58	0·95	0·65	21	44	64
0·031	7·38	6·46	0·51	0·33	25	45	69

[1] $c_E = c_{Na}$ is the number of pectin or sodium equivalents per litre of solution, $\varkappa_{Na}$ the specific conductivity of the sodium ions calculated by means of the equation $\varkappa_{Na} = c_E \cdot \Lambda_{Na} \cdot 10^{-3}$, where Λ_{Na} is the ionic equivalent conductivity of the sodium ions at the concentration in question as given by Kohlrausch (Landolt-Börnstein, Tabellen), and $u_k = (\varkappa - \varkappa_{Na})/c_E \cdot 10^{-3} \cdot 96\,500$, the mobility of the pectin anion.

Table 18.[1] — Neutralized Citrus Pectin. — Temp.: 20° C.

Concentration		pH	$\varkappa \cdot 10^4$	$\varkappa_{Na} \cdot 10^4$	$u_k \cdot 10^5$	Λ_{Na}	Λ
per cent	$c_E \cdot 10^4$						
0·833	123	6.65	6·10	5·17	8	42	50
0·417	61·50	6·89	3·26	2·64	10	43	53
0·208	30·75	6·58	1·72	1·35	12	44	56
0·104	15·38	6·51	0·91	0·68	16	44	59
0·052	7·69	6·37	0·48	0·35	18	45	62
0·026	3·85	6·21	0·27	0·17	27	45	70
0·013	1·92	6·13	0·14	0·09	27	45	73

Table 19.[1] — Neutralized Gum Arabic. — Temp.: 20° C.

Concentration		pH	$\varkappa \cdot 10^4$	$\varkappa_{Na} \cdot 10^4$	Λ_{Na}	Λ
per cent	$c_E \cdot 10^4$					
1·000	83·0	7·65	3·07	3·57	43	37·0
0·500	41·5	7·61	1·50	1·78	43	36·1
0·250	20·8	7·17	0·76	0·92	44	36·5
0·125	10·4	7·01	0·40	0·46	44	38·5
0·0625	5·2	6·84	0·23	0·23	45	44·2

implies as usual an increase in the degree of ionization, i. e. an increase in the number of charges of the particles. In addition, however, we probably have to take into account in solutions of these substances the action of intermolecular forces, varying in strength with the degree of ionization. It is thus probable that the particles can move more freely in a neutral solution than they can in an acid one. The reader may be reminded here of the fact that the jellying power, for instance, asserts itself only in acid media.

The behaviour of the sodium gum is rather bewildering. The relation between $\varkappa$ and $\varkappa_{Na}$ (Table 19) is quite analogous to that between $\varkappa$ and $\varkappa_H$ in the case of the acid substance. Yet, measured electrophoretically (Table 21), the mobility of the gum anion in neutral solution is quite comparable with that of the pectin anion. The pheno-

[1] The symbols are those used and explained in Table 17.

menon may be accounted for by assuming the degree of ionization to be less than 100 per cent. This would be in accordance with results obtained by Oakley (81) from osmotic measurements on sodium gum, according to which the degree of ionization may vary from 30 to 68 per cent as the concentration decreases from 1 to 0·1 per cent. On the other hand, too much importance should not be attached to these figures owing to the fact that the osmotic activity of the compensating ions is always smaller than their electrochemical activity. This fact is also recognized by Oakley, who mentions the possibility of surface conductance.

Briggs (79), wo obtained results quite analogous to those recorded in Table 19, considers the cations to act as conductors for almost all the current, and assumes the salts to by ionized to a very great extent. In consequence the mobility of the colloid anion is assumed to be very low. To judge from the electrophoretic measurements given below, however, such an assumption does not seem to be quite justified.

The similarity in conductivity of the acid and the neutralized gum also manifests itself in the fact that the equivalent conductivity of the neutralized substance, calculated on the basis of complete ionization, exhibits a minimum, as did the acid gum, and, moreover, as is shown in Fig. 13, both minima occur at practically the same equivalent concentration. No such minimum was found in the case of the neutralized pectins.

ELECTROPHORETIC MOBILITY OF THE POLYURONIDE ANIONS.

The question arises, are the polyuronide anions in general to be regarded as free ions and, if so, what mobility and transference number may be attributed to them? The authors quoted above give no experimental data on these questions. It seemed worth while, therefore, to make some electrophoretic measurements in order to gain some knowledge on the subject.

The method used was the moving boundary method as worked out by Tiselius and co-workers (82, 83). The results are given in Tables 20—21 and Fig. 14. u_k is the mobility of the colloid anion in cm^2 sec.volt. The descending and the rising boundary in most cases gave concordant values of u_k. In cases where conflicting values were ob-

Table 20. — Mobility of the Pectin Anions at 20° C.

No.	Conc. per cent	Buffer[1]	pH	Ionic strength	$u_k \cdot 10^5$		
					Apple pectin	Citrus pectin	Beet pectin
1	0·30	P	7·0	0·1	15·9	9·9	12·1
2	»	A	5·0	»	11·1	7·9	9·9
3	»	»	4·0	»	8·8	7·4	8·2
4	»	»	3·3	»	6·3	6·0	6·7
5	»	C	2·8	»	3·8	3·3	4·5
6	»	»	2·9	0·05	4·4	—	—
7	»	»	2·9	0·015	7·1	—	—
8	0·15	»	2·9	»	6·9	—	—
9	»	HCl + Na-Citr.	2·2	»	1·3	—	—
10	»	P	7·3	»	22·7	—	—
11	»	SX[2]	9·9	»	38·8	—	—
12	0·10	P	6·7	0·01	23·4	16·6	—
13	»	A	5·0	»	24·6	—	—
14	»	C	3·1	»	9·8	—	—
15	»	P	5·8	0·001	44	—	—

tained, i. e. at very low ionic strengths, u_k refers to the descending boundary as giving a more accurate value.

Since the method requires buffered solutions, the charge of the particles is certainly reduced and hence the mobility recorded is lower than that which might be expected in a salt-free solution. A comparison of No. 1 and 10, or 5, 6 and 7, or 12 and 15 in Table 20, referring to apple pectin, or No. 2—5 in Table 21, for gum arabic clearly shows that decreasing ionic strength brings about increasing mobility. Particularly at the very low ionic strengths ranging from 0·01 to 0·001, the mobility seems to increase strongly, attaining a value of the magnitude $40 \cdot 10^{-5}$ cm²/sec.volt in neutral solution. — The dependence of u_k upon ionic strength is shown diagrammatically in Fig. 14 *B*.

As may be seen from the Tables and Fig. 14 the electrophoretic

[1] P means phosphate buffer, A acetate, C citrate, and SX soda-borax buffer.

[2] In this buffer the methoxyl groups were split off and the mobility refers to the pectic acid anion.

Table 21. — Mobility of the Gum Arabic Ions at 20° C.

No.	Conc. per cent	Buffer	pH	Ionic strength	$u_k \cdot 10^5$
1	0·5	P	6·8	0·01	19·6
2	0·1	»	6·8	0·05	19·1
3	»	»	6·9	0·01	19·4
4	»	»	6·5	0 005	20·1
5	»	»	7·2	0·001	36
6	»	PX [1]	8·4	0·01	23·4
7	»	P	5·3	»	20·0
8	»	C	3·6	»	18·3
9	»	»	3·0	»	11·7
10	»	0·005 N-$Co(NH_3)_6Cl_3$	4·7	»	3·4
11	»	0·005 N-$K_4Fe(CN)_6$	6·7	0·013	32·3
12	»	0,005 N-$Th(NO_3)_4$ [2]	3·2	0·013	15·7
13	0·2	0·001 N-$Th(NO_3)_4$	3·3	0·003	5·4

mobility is dependent mainly upon the hydrogen ion concentration, as was to be expected, and upon the valency of the ions constituting the medium. The concentration of the substance seems to be of less importance. At a pH of about 2 the mobility of both pectin and gum anions approaches zero (Fig. 14). The acid solutions used for conductivity measurements (Tables 14—16) had a pH ranging from 2·4 to 4·2. From No. 3 and 14 in Table 20, and No. 8 and 9 in Table 21 we might conclude, therefore, that the pectin anions in these solutions have a mobility of the order of $10 \cdot 10^{-5}$ cm²/sec.volt, and the gum anions a somewhat higher value. It seems reasonable, then, that the hydrogen ions should account for practically all the current transport in these solutions. Assuming the mobility of the hydrogen ion to be that at infinite dilution, the transference number of the colloid anion would be of the magnitude 0·03—0·05.

In neutralized solutions (sodium pectinate, sodium gum), on the

[1] PX means phosphate-borax buffer. The other symbols are the same as in Table 20.

[2] In this medium the gum particles assumed a positive charge as was apparent from a reversal in the direction of migration. The solution had a milky appearance ('autocomplex coacervation', according to Bungenberg de Jong, 84). In experiment No. 13 the solution was quite clear, but became easily turbid when diluted with the buffer.

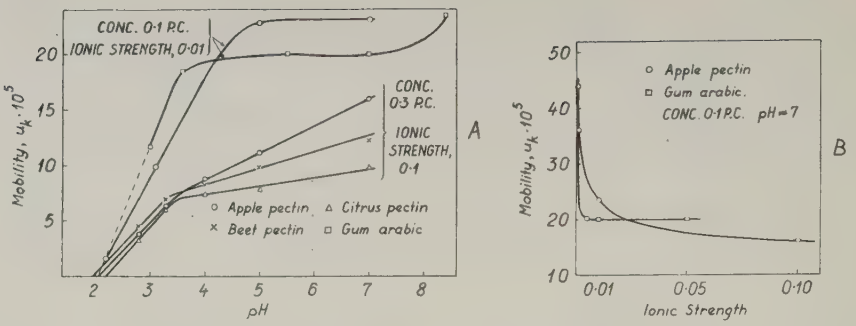

Fig. 14.—Variation of the mobility of the polyuronide anions with pH and ionic strength.

other hand, the mobility of the colloid anions is considerably higher (No. 10 and 15 in Table 20, No. 5 in Table 21), in some cases being comparable to that of the sodium ion (i. e. about $47 \cdot 10^{-5}$ cm^2/sec.volt at 20° C and infinite dilution). As was shown above this fact is also in accordance with the value for the conductivity of the pectinate solutions. One might expect that the mobility of the gum anions, too, should manifest itself in the conductivity of the solutions of sodium gum. In addition to the possible reasons given above for the anomalous behaviour of this substance the fact should be mentioned that the size of the gum particles is considerably larger than that of the pectin particles, as will be shown later.

The markedly antagonistic effect of positively and negatively charged multivalent ions upon the mobility of the gum particles is illustrated by experiments No. 10—13, Table 21. The mobility in a 0·005 N-solution of cobaltichloride hexammine is only $3{\cdot}4 \cdot 10^{-5}$ cm^2/sec. volt, while in an equally dilute solution of potassium ferrocyanide it amounts to $32{\cdot}3 \cdot 10^{-5}$ cm^2/sec.volt, i. e. a value of the magnitude determined in a phosphate buffer of the same pH and an ionic strength ten times lower. The effect of the negatively charged multivalent ions upon the gum anions is evidently equivalent to that of a decrease in ionic strength, i. e. an attenuation of the ionic atmosphere or diffuse double layer surrounding the particles, and hence an increase in their effective charge. The effect of the positively charged multivalent ions is the reverse. In a thorium nitrate solution of suitable concentration the effective charge may be brought up to zero or even made positive, a fact which might be inferred from the last two experiments recorded in Table 21.

As will be shown later the antagonistic valency effect is also clearly reflected in the values of the sedimentation and the diffusion velocity of both gum arabic and pectins.

APPARENT DISSOCIATION CONSTANT OF THE ACID SUBSTANCES.

The titration curves for the acid polyuronides strongly resemble those obtained with fairly strong monobasic acids. An example is given in Fig. 15 *A*, referring to commercial apple pectin. Similar curves were obtained by Thomas und Murray (85) and by Briggs (79) for gum arabic. The carboxyl groups are probably spaced in such a way as to exert no noticeable influence upon one another's dissociation. The acids may, therefore, be looked upon as monobasic acids with an apparent dissociation constant k (cf. Pauli and Valkó, 61, p. 111 ff.). In order to get an idea of the strength of the acids, values of k were calculated by applying the mass action law to the equilibrium (10). p. 41.

$$k = \frac{c_H^2}{c_E - c_H}, \qquad (11)$$

where the notations are those explained in Table 14. As may be seen from Tables 14—16 and from Fig. 15 *B*, k is no real constant. It seems to attain a constant value only at higher concentrations. With increasing dilution the 'constant' rapidly decreases. From similar results of measurements on gum arabic, Briggs (79), assumes 'a reversible peptization of the micella' when the solution

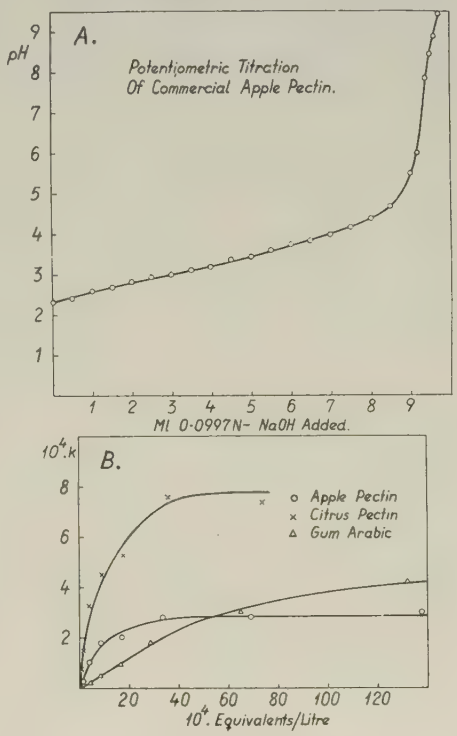

Fig. 15.—*A*. Potentiometric titration of commercial apple pectin, starting with 25 ml 1·66 per cent solution.

B. Variation of the dossociation 'constant' k of the acid polyuronides with equivalent concentration.

is diluted and 'that this is accompanied by a marked decrease in the dissociation tendency of the acid groups present on the micella'. Such an interpretation was put forward earlier by Rabinowitsch and Laskin (86) in the case of colloidal silicic acid. On the other hand, neither osmotic measurements (Oakley, 81) nor the evidence obtained in sedimentation and diffusion experiments (cf. above p. 44) are in accordance with this interpretation in the case of gum arabic and pectins.

SEDIMENTATION MEASUREMENTS.

METHOD.

The behaviour of the acidic polyuronides on sedimentation in strong centrifugal fields has been studied with the aid of the ultracentrifuge developed by Svedberg and co-workers. For details concerning the construction and operation of the ultracentrifuge the reader is referred to Svedberg and Pedersen's comprehensive monograph 'The Ultracentrifuge' (Oxford 1940) [Die Ultrazentrifuge, (Dresden und Leipzig 1940)][1] and to several papers by Svedberg (87). Only the main features of the procedure and some definitions will be given here.

The solution to be studied is enclosed in the sector-shaped cavity of a cylindrical cell provided with quarts windows for observation. Only about 0·6 ml of solution is required. The cell is fixed in the centrifuge rotor at a distance of about 6 cm from the centre of rotation. The rotor is driven by means of turbines and oil. A maximum number of revolutions of about 70 000 R.P.M., and a centrifugal force some 350 000 times gravity may be attained.

The course of the sedimentation is followed in most cases by means of the so called scale method worked out by Lamm (88). A finely ruled transparent scale is photographed through the rotating cell. The resulting photograph reveals the position of any boundary formed by sedimenting molecules as a scale line displacement, caused by the variation of refractive index within the boundary range. The scale line displacement is measured against a reference scale, i. e. a scale photograph obtained from a run with the cell filled with solvent, other things being equal. The magnitude Z of the scale line displacement is proportional to the concentration gradient dc/dx at a distance x from the centre of rotation,

$$Z = G\,a\,b\,\alpha \cdot dc/dx \qquad (12)$$

[1] Referred to below as UC.

where G is an enlargement factor, a the length of solution traversed by the light, b the distance between the scale and the middle of the cell, and α the refractive index increment dn/dc of the solute. Plotting Z against x we get a sedimentation diagram. In such a diagram every boundary formed during the run will appear as a maximum. From a series of sedimentation diagrams the sedimentation velocity dx/dt for a certain molecular species can be calculated. Reducing this velocity to a value corresponding to a centrifugal field of the strength 1 dyne, we get the sedimentation constant s, defined by the equation

$$s = \frac{dx}{dt} \Big/ \omega^2 x, \tag{13}$$

where ω is the angular velocity of the rotor, and x the mean distance of the boundary from the centre of rotation. The sedimentation constant is expressed in the unit 10^{-13} cm/sec. dyne, called one Svedberg and denoted by S. The sedimentation constant is usually reduced to the value it would assume if pure water at 20° C were used as a solvent. This value is obtained by multiplying the constant by a factor involving viscosity, density and partial specific volume data. The reduced sedimentation constant will be denoted by s_{20}.

The sedimentation constant is an important factor in the Svedberg formula for calculating the molecular weight,

$$M = \frac{RTs}{D(1 - V\varrho)}, \tag{14}$$

where R is the gas constant, T the absolute temperature, D the diffusion constant and V the partial specific volume of the solute, and ϱ the density of the solvent.

In order to obtain conveniently measurable sedimentation velocities of pectic substances and alginic acid in aqueous solution, it was found necessary to use centrifugal fields of the highest possible intensity, i. e. a rotor speed equal to 70 000 R.P.M. For the gums 40 000 R.P.M. proved to be the most suitable number.

The scale photographs were taken with mercury light of wave length 546 mμ ('Wratten filter 77') and an 'Eastman Spectroscopic Plate, Type IV G' or with light of wave length 436 mμ (Aughey and Lansing's β-methyl-esculetin filter) and a 'Cramer Super Contrast Plate' or 'Agfa Spectral blau, extra hart'.

SIGNIFICANCE OF THE SEDIMENTATION CONSTANT.

The appearance of the sedimentation diagrams of the various polyuronides may be seen in Fig. 17. There is only one maximum at any given time. The sedimentation constant is calculated throughout from the change in time of the position of the maximum point of the curve. Starting from the fact that the substance in question forms a poly-

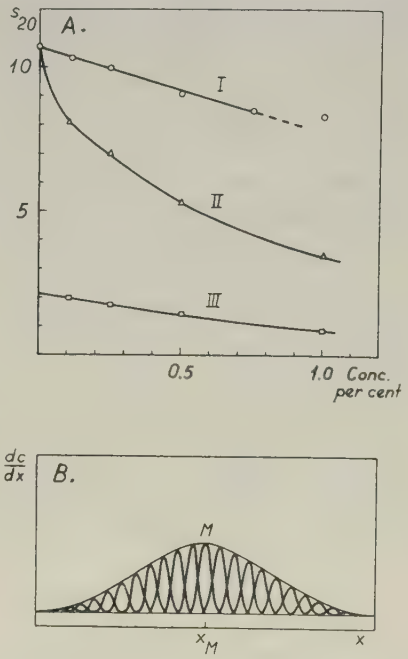

Fig. 16.—*A*. Variation of s_{20} with concentration. I. Gum arabic in 0·2 N-NaCl. II. Gum arabic in a mixture containing equal amounts of gum and commercial apple pectin in 0·2 N-NaCl. III. Pectin in the mixture.

B. Schematic picture exhibiting the manner in which the sedimentation curve is 'built up' in the case of a polydisperse substance at low concentration. The 'average sedimentation constant' is calculated from the change of position of the maximum point *M*.

disperse system in solution, it may be asked what significance should be attributed to a sedimentation constant calculated in this way. For higher concentrations we can not give any clear significance to the sedimentation constant. The large particles, for instance, tend to sediment faster than the small ones, but their speed is reduced, particularly in the case of elongated particles, on account of the strong friction against the surrounding smaller particles (cf. Fig. 16 *A*). The latter, on the other hand, tend to lag behind. Their speed is increased, however, since dilution implies decreasing frictional resistance, a fact which is evident from the course of the curves in Fig. 18. In consequence the boundary between solvent and solution becomes abnormally sharp (cf. Fig. 17).

On dilution the possibility of independent sedimentation increases, and the curves broaden noticeably as the time increases. As a matter of fact, this broadening has been made use of in the gradation of polydispersity (Gralén, 89). At a dilution sufficiently high as to permit of completely free sedimentation of the individual particles the sedimentation curve may be regarded as the resultant of a series of individual curves (Fig. 16 *B*), and it is evident, provided that the influence of diffusion may be neglected, that the maximum point will be determined finally by molecules or particles of a single magnitude, viz. those of the highest frequency in the particle size distribution. The sedimentation constant for zero concentration is thus to be regarded as belonging to this one class of particles, a fact which should be remembered when calculating the molecular weight. It appears from the diagrams in Fig. 18, that the extrapolation of s_{20} to zero concentration offers no difficulties.

If we may assume the sedimentation curves at the lowest concentrations to represent the particle size distribution, we may judge from the shape of the curves to what extent the size of the particles belonging to the maximum are to be regarded as an average of the whole series of particle sizes. From the rather symmetric curves for the polyuronides in a suitable electrolytic medium (Fig. 17) it might therefore be inferred that the maximum represents with good approximation a weight-average molecular weight (cf. p. 97).

CHARGE EFFECTS.

The course of the sedimentation of the polyuronides in distilled water and in salt solutions is elucidated by the curves in Figs. 17—18 and by the data in Table 22. As may be seen from No. 9, 20 and 21 in Table 22, the sedimentation velocity in pure water is considerably smaller than that in an electrolytic medium. This fact is due to retarding electric forces set up whenever charged particles move in a pure solvent.

Pectins at a cencentration of 0·3 per cent will hardly sediment at a measurable velocity in pure water. In some experiments with acid and neutralized apple pectin, the substance had not come off the meniscus even after a four hours' run at the highest speed of the rotor. For comparison, purified methyl cellulose was run under the same conditions. The sedimentation velocity of this uncharged sub-

TABLE 22. — DEPENDENCE OF s_{20} UPON CONCENTRATION AND MEDIUM.

No.	Preparation[1]	Medium:	Conc. per cent							
			0·30	0·20	0·15	0·10	0·05	0·03	0·01	Zero
1	Free apple pectin . .	0·2 N-NaCl	1·40	1·62	1·79	2·10	2·50	—	—	2·8
2	Apple pectin acc. to v. Fellenberg . . .	» »	1·55	1·70	1·76	1·83	1·90	—	—	2·0
3	Commercial apple pectin, acidic	» »	1·55	1·69	1·80	1·92	2·03	—	—	2·2
4	Do. neutr. with NaOH	» » , pH 7[2]	1·53	1·67	1·74	1·87	1·92	—	—	2·0
5	Pectic acid from comm. apple pectin . . .	0·2 N-Na_2CO_3	1·53	1·68	1·77	1·82	1·90	—	—	2·0
6	Free citrus pectin . .	0·2 N-NaCl	1·42	1·58	1·90	2·32	3·03	3·40	3·8	4·0
7	Extracted citrus pectin	» »	1·57	1·68	1·82	1·93	2·07	—	—	2·2
8	Beet pectin	» »	1·72	1·79	—	1·82	—	—	—	1·9
9	Comm. apple pectin Extr. citrus pectin Beet pectin	H_2O	—	—	0·3	—	0·8—1·2	—	—	—
10	Red currant pectin . .	0.2 N-NaCl	1·54	—	1·73	1·85	—	—	—	2·0
11	Carrot pectin	» »	1·58	1·73	—	1·83	—	—	—	2·0
12	Flax pectin (A) . . .	0·2 N-$(COONH_4)_2$	1·55	1·70	—	1·85	1·93	—	—	2·0
13	Alginic acid	0·2 N-Na_2CO_3	—	1·62	1·80	1·97	2·18	2·26	—	2·4
14	Do., commercial . . .	» »	1·43	—	1·86	2·10	2·12	2·69	—	3·0

	Gum arabic	Medium:	Conc. per cent						
			1·00	0·75	0·50	0·25	0·12	0·06	Zero
15	Acid gum	0·2 N-NaCl	8·3	8·5	9·1	10·0	10·3	—	10·7
16	Do.	0·2 N-NaCl + HCl, pH 1.7	—	8·9	9·8	10·7	—	—	11·5
17	Do.	0.2 N-$CaCl_2$	—	8·6	9·4	9·9	—	—	10·7
18	Do.	0.2 N-La $(No_3)_2$	—	8·8	9·4	10·1	—	—	10·7
19	Sodium gum	0.2 N-NaCl + NaOH, pH 7	7·7	8·2	8·9	9·5	—	—	10·4
20	Acid gum	H_2O	3·5	4·0	4·5	5·0	6·2	6·9	—
21	Sodium gum	»	—	—	5·2	5·7	—	6·0	—

[1] Cf. p. 16.

[2] pH adjusted to 7 with NaOH.

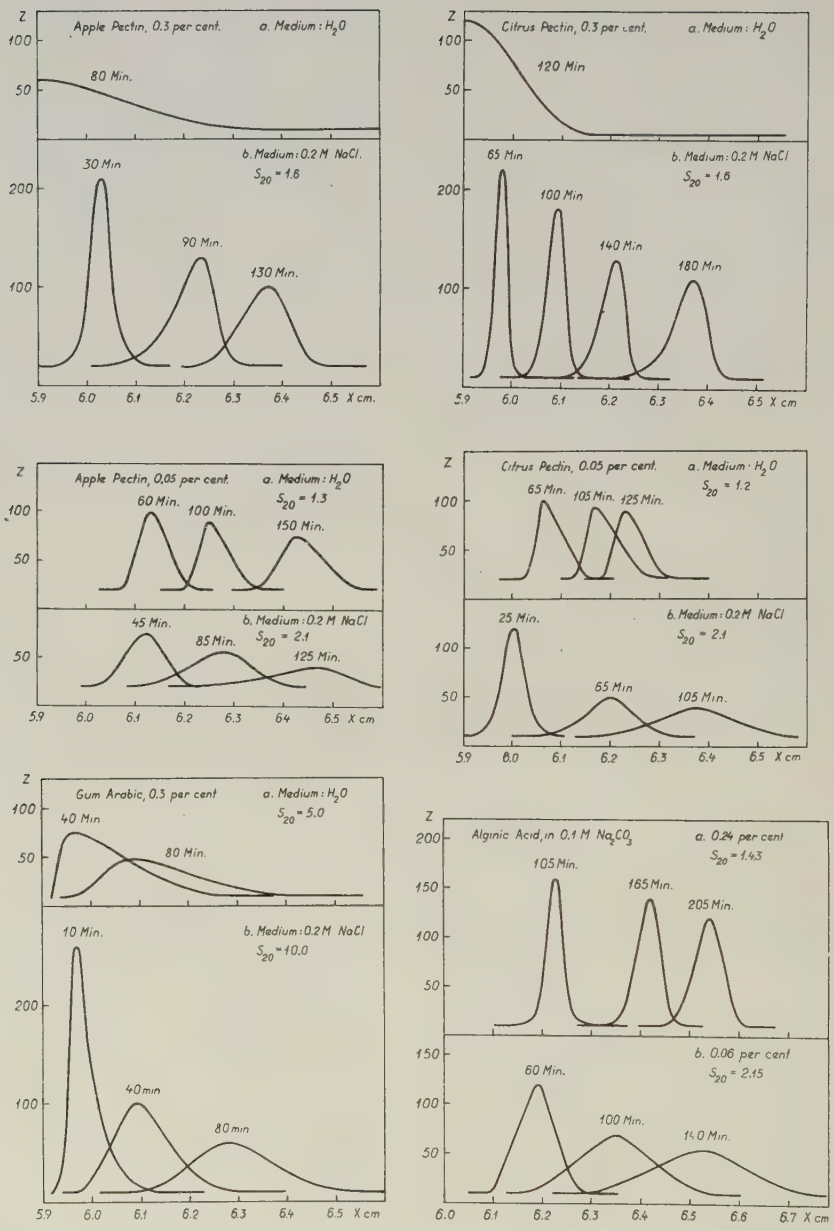

Fig. 17. — Sedimentation diagrams for the polyuronides in various media at various concentrations.

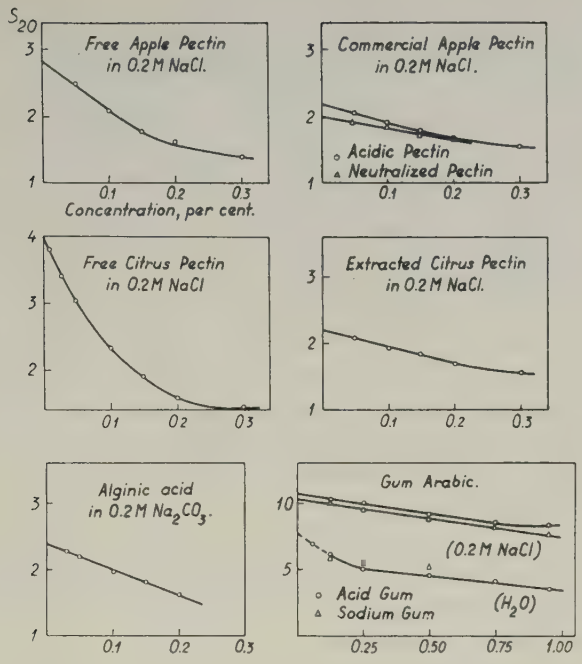

Fig. 18.—Variation of s_{20} with concentration.

stance was the same in water as in 0·2 N-NaCl, corresponding to a sedimentation constant of 1·1 S at 20° C.

At lower concentrations the sedimentation velocity of the pectins in water becomes measurable. Thus at a concentration of 0·05 per cent — below this concentration accurate sedimentation measurements could hardly be made — sedimentation constants of the order 0·8—1·2 S were calculated for the type pectins, whether acid or neutralized. The sedimentation velocity in 0·2 N-NaCl with the same concentration of the pectins corresponds to sedimentation constants of the order 2 S. The marked dependence of the sedimentation velocity upon concentration of a neutral salt (NaCl) at higher concentrations also is clearly shown in Table 23 and Fig. 19. As may be seen the value of the sedimentation constant rapidly increases up to a salt concentration of about 0·05 N and remains practically constant on further addition of salt.

The diagrams for gum arabic in pure water exhibit the formation of a boundary at higher concentrations also. A measurable sedimentation takes place. The sedimentation velocity is reduced again, how-

Table 23. — Dependence of s_{20} upon Concentration of NaCl.

NaCl, Molarity	0·3 per cent			0.5 per cent
	Apple pectin	Citrus pectin	Beet pectin	Gum arabic
0·001	0·8	1·0	1·0	4·9
0·005	1·2	1·3	1·3	7·1
0·01	1·3	1·4	1·4	7·8
0·05	1·6	1·6	1·5	8·7
0·1	1·6	1·6	1·5	8·8
0·2	1·6	1·6	1·5	9·1
0·4	1·6	—	—	—
1·0	1·6	—	—	—

ever, on an average to about half its value in 0·2 N-NaCl (Fig. 18). The reduction is higher for the acid substance than for the neutralized one, it is also higher the higher the concentration of the gum.

The influence of the charges on the sedimentation of colloidal electrolytes in strong centrifugal fields has been treated theoretically by Svedberg (90), Tiselius (91), and Lamm (92). Their reasoning is based on the assumption that the colloidal electrolyte is completely dissociated and that the colloidal ion acts as a free ion. Moreover the conditions are considered only in that part of the solution where the concentration of the solute is to be assumed constant, i. e. any charge effects at the actual boundary between solvent and solution or at the bottom of the cell are disregarded.

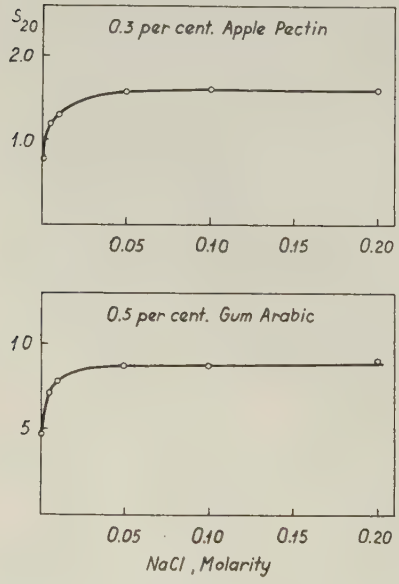

Fig. 19. — Variation of s_{20} with concentration of NaCl.

As a result of the theoretical considerations Eq. (15) was established (Tiselius, 91). This equation gives the relation between the actual sedimenta-

tion constant s'_k of the colloidal ion and s_k, that which would be obtained if the particle carried no charges.

$$s'_k = s_k \left(1 - F \frac{n c_k u_k}{\varkappa}\right) = s_k (1 - t_k) \qquad (15)$$

Here n is valency, c_k number of mols per c.c., and u_k mobility of the colloidal ion, $\varkappa$ is the specific conductivity of the solution, F the Faraday constant, and t_k the transference number of the colloidal ion. For the equation to be valid, it is necessary to be able to neglect the sedimentation of the small amounts of low molecular electrolytes present.

The principles underlying this equation seem to hold good at least in the case of certain proteins, such as phycoerythrin at pH 8, with sodium ions as compensating ions, as was shown by Tiselius (91). In the case of the polyuronides, on the other hand, the conception of free ionic migration can not be maintained as far as acid substances are concerned. It was shown above, from measurements of both conductivity and electrophoresis, that the transference number of the supposed colloid anions in solutions of the acid substances must be very low, possibly of the order 0·03—0·05, probably still lower, perhaps zero. According to Eq. (15) we should thus expect s'_k to be practically identical with s_k. In reality the value of s'_k does not amount to 0·5 s_k in measurements at the highest concentrations in Table 22, while at the lowest concentrations s'_k is of the magnitude 0·6 s_k, s_k being taken as the value obtained from sedimentation in 0·2 N-NaCl.

On exchanging the hydrogen ions for sodium ions by neutralization, the ratio s'_k/s_k is changed only slightly, its value for the lowest concentrations still being of the magnitude 0·6, thus corresponding to a value of t_k equal to about 0·4. In this case, however, if we may judge from the electrophoretic measurements, it is quite possible that the celloid anion might have the right mobility to satisfy the t_k-value. In a 0·1 per cent solution of sodium gum, for instance, at an ionic strength equal to 0·001, the gum was found to have the mobility $u_k = 36 \cdot 10^{-5}$ cm²/sec. volt at 20° C (Table 21, No. 5) while according to Kohlrausch (Landolt-Börnstein, Tabellen) the sodium ion at the corresponding equivalent concentration and temperature has a mobility of about $46 \cdot 10^{-5}$. From these figures $t_k = 0{\cdot}44$ is calculated. Similar relations hold for sodium pectinate (Table 20, No. 15).

The different behaviour of the gum anions as regards conductivity on the one hand and electrophoresis on the other (p. 46) should not be ignored. Yet, on account of the rather similar conditions in electrophoresis and sedimentation as compared with those in conductance measurements, i. e. the migration of the particles in one direction in a constant electric field, whether applied externally or set up through the motion of the particles themselves, it seems reasonable to attach more importance to the evidence obtained in electrophoretic experiments than to those obtained from conductivity.

Table 24. — Influence of the Charges upon Sedimentation Velocity.

Substance	pH	$z \cdot 10^5$	$u_k \cdot 10^5$	s'_k	s_k	s'_k/s_k	M
Phycoerythrin	8·1	2·9	25·4	6·7	11·6	0·58	290 000
Sodium gum, 0·1 per cent . . .	7·0	3·3	36	5·9	10·2	0·58	260 000

Some figures are recorded in Table 24 in order to show how very similar is the influence of the charges on the sedimentation of sodium gum and phycoerythrin. The data referring to phycoerythrin are those given by Tiselius (91). M is molecular weight.

Although the charge effects in the case of the neutralized substances may possibly be interpreted, therefore, on the basis of the principles underlying Eq. (15), i. e. by considering the probable conditions within the bulk of the solution, evidence will be given later, in connection with the investigation of the diffusion phenomena, indicating the possibility that the reduction of the sedimentation velocity as it is actually measured may be due partly or perhaps mainly to the very strong diffusion potential set up at the boundary.

Attention should be drawn here only to the fact that the sedimentation curves of sedimentation in pure water always slope gradually towards the solution side while they are rather steep towards the solvent (Fig. 17). This may be looked upon as the result of the cooperation of the diffusion potential and the centrifugal force, the particles within the bulk of the solution sedimenting faster than those in the boundary range, a fact that is most clearly shown by the curves referring to cases where the substance cannot leave the meniscus (Fig. 17). This means that the migration of the particles determining

the maximum of the sedimentation curve, and hence the sedimentation constant, does not give an accurate value for the average sedimentation velocity of the substance.

SEDIMENTATION IN MEDIA CONTAINING EXCESS OF ELECTROLYTE.

It was assumed above that the use of 0·2 N-NaCl as a medium would imply the elimination of the charge effects and make possible the determination of s_k. As was shown by Lamm (92), this is not strictly true. Even in the presence of a salt AB at a concentration c which may be considered large as compared with the concentration c_1 of the colloidal electrolyte $A_n K$, a charge effect will remain, reducing the sedimentation velocity so as to correspond to the sedimentation constant s_1 instead of s_k. s_1 is expressed by the following equation:

$$s_1 = \frac{M_1(1 - V_1\varrho)\left[f_A + \left(1 + \frac{n c_1}{c}\right) f_B\right] - n M (1 - V\varrho) f_A}{f_K\left[f_A + \left(1 + \frac{n c_1}{c}\right) f_B\right] + \frac{n^2 c_1}{c} f_A f_B}, \qquad (16)$$

where f is the frictional force per gram ion of the species indicated, M_1 the molecular weight, and V_1 the partial specific volume of the colloidal electrolyte, M and V the corresponding magnitudes for the salt, n the number of effective charges of the colloidal ion, and ϱ the density of the solution.

In the case of neutralized commercial apple pectin (sodium pectinate) at a concentration of 0·3 per cent in 0·2 N-NaCl as a medium, for instance, we may estimate the reduction of the sedimentation velocity due to the remaining charge effect on the assumption that f_{Na} is equal to f_{Cl} and from the figures given below, some of which are taken from determinations to be described later on. We have $M_1 = 50\,000$ and $V_1 = 0{\cdot}6$ approximately. Furthermore $M = 58{\cdot}5$, $V = 0{\cdot}3$ (as calculated from the density of sodium chloride solutions at various concentrations) and ϱ roughly equal to 1. With the concentrations expressed in the proper unit, i. e, gram ions per c.c., $c_1/c = 3 \cdot 10^{-4}$. A maximum valency $n_{max} = 115$ may be calculated from the equivalent weight, 435, and the average molecular weight of the pectin. Owing to the effect of the ionic atmosphere surrounding the

particles in the salt solution the number of *effective charges* n_e is certainly far below that value, but even if n is put equal to n_{max} the value of nc_1/c amounts to only about 0·03 and may be neglected as compared with 1. From the data given we thus obtain

$$\frac{s_1}{s_k} = \frac{1 - n \cdot 10^{-3}}{1 + 1{\cdot}5 \cdot 10^{-4} n^2 f_{Na}/f_K}. \tag{17}$$

Assuming free ionic migration we have, $nf_{Na}/f_K = u_k/u_{Na}$, a ratio that might be of the order of $^1/_3$. Putting this value in Eq. (17) we finally obtain

$$\frac{s_1}{s_k} = \frac{1 - 10^{-3} n}{1 + 0{\cdot}5 \cdot 10^{-4} n}. \tag{18}$$

For $n = n_{max} = 115$, a maximum reduction of the sedimentation velocity by about 10 per cent is calculated. As has been mentioned above, however, the number of effective charges n_e is certainly far less than n_{max}. Proceeding along the lines indicated by Tiselius and Lamm (91, 92), i. e. assuming the molar frictional constant f_K for the colloidal ion in a given solution to have the same value in electrophoretic motion as in sedimentation the magnitude of n_e may be estimated. According to Eq. (16) we have approximately

$$f_K = \frac{M_1(1 - V_1\varrho)}{s_1}. \tag{19}$$

Furthermore

$$f_K = \frac{n_e F \cdot 10^7}{u_k}. \tag{20}$$

Eliminating f_K we obtain

$$n_e = \frac{u_k M_1 \cdot (1 - V_1\varrho)}{s_1 F \cdot 10^7}, \tag{21}$$

an equation that is also to be found in Tiselius' paper quoted above.

For a 0·3 per cent solution of the sodium pectinate in a phosphate buffer of ionic strength 0·1 and pH 7, u_k is equal to $16 \cdot 10^{-5}$ (Table 20 No. 1) and $s_1 = 1{\cdot}5$ S. With these values and those given above $n_e = 22$ may be calculated. If we may assume that the pectin anions carry an effective charge of this magnitude in 0·2 N-NaCl also the sedimentation velocity would according to Eq. (18) be reduced by about 2 per cent.

Similar calculations applied to sodium gum in 0·2 N-NaCl show that the reduction of the sedimentation velocity for this substance is still lower than in the case of the pectin.

It is interesting to note in this connection that the sedimentation velocity of both pectin and gum is distinctly, though not much, lower in a neutral solution of 0·2 N-NaCl than it is in an acide one. (Cf. Table 22, No. 3 and 4, 15 and 19). This difference may be partly due to charge effects of the kind dealt with here.

TABLE 25. — VARIATION OF s_{20} FOR GUM ARABIC IN VARIOUS MEDIA.
Conc.: 0·5 per cent.

Normality	Medium		
	$K_4Fe(CN)_6$	$Co(NH_3)_6Cl_3$	$Th(NO_3)_4$
0·001	5·2	6·9	6·6
0·010	6·6	8·9	9·7
0·050	7·9	9·1	—
0·100	7·9	9·3	10·5

In other cases also the sedimentation velocity seems to be influenced by the charges, even in the presence of considerable amounts of a salt. This is evident from the figures given in Table 25, referring to 0·5 per cent solutions of gum arabic in various electrolytic media. In a 0·1 N-solution of potassium ferrocyanide, for instance, the sedimentation velocity is about 20 per cent lower than in a thorium nitrate solution of equal concentration. As was shown above (p. 50), the effective charges of the gum particles in the two media are certainly of different magnitudes. The possibility should not be precluded, however, that the higher sedimentation velocity in the thorium nitrate solution may be partly due to an adsorption of thorium ions on the surface of the gum particles, thus increasing their mass. Such an adsorption is in accordance with the fact that the negatively charged gum particles may assume a positive charge in a thorium nitrate solution of suitable concentration (Table 21, No. 12).

The variation of s_{20} with the concentration of gum arabic in some other electrolytic media is shown in Table 22. Exchanging NaCl for $CaCl_2$ or $La(NO_3)_3$ of the same equivalent concentration brings about a slight increase in the sedimentation constant at higher concentra-

tions of gum. On extrapolation to zero concentration, however, the resulting constant seems to have the same value as that obtained for acid gum in 0·2 N-NaCl.

In a markedly acid solution of 0·2 N-NaCl (pH 1·7) the sedimentation velocity is distinctly higher than in the other media. This fact is in accordance with the results reached by Oakley (46) in his osmotic measurements on gum arabic. Using 0·02 N-HCl as a medium, he found a value of π_0, i. e. osmotic pressure in mm water/concentration in per cent, for zero concentration, equal to 8·8, while with 0.1 N-NaCl, π_0 had the value 10·3. According to Oakley the discrepancy may be accounted for in two different ways. Either one may assume that the gum particles aggregate and that the aggregation varies under the different experimental conditions, or else the discrepancy may be ascribed to varying degrees of suppression of the ionic pressure difference due to Donnan equilibrium. For the moment it is not possible to decide definitely what is the reason for the different behaviour of the gum in acid and neutral solution.

Another peculiarity found by Oakley (47) might be mentioned here. In his measurements of the osmotic pressure of sodium gum in 0·1 N-NaCl, he found a rather sudden decrease in the values of P/C (pressure/concentration) within the concentration range 0·30—0·15 per cent. A similar decrease also appeared in the case of calcium gum in 0·1 N-$CaCl_2$ at a concentration of about 0·5 per cent. In both cases there was a drop in the value of P/C from about 13 to about 10. Owing to the fact that the osmotic measurements at a concentration below 0·3 per cent were found to be impaired by rather large errors, Oakley proposed that the discussion of these peculiarities should be postponed until the osmotic pressure measurements at the lowest concentrations had been confirmed by means of the ultracentrifuge.

The present author found no corresponding change in the sedimentation velocity at the concentrations mentioned. In this connection it should be emphasized, however, that the measurements of the sedimentation velocity of gum at concentrations below 0·1 per cent or thereabout are rendered impossible by a very rapid blurring of the boundary between solvent and solution.

The influence of the charges upon the sedimentation of apple pectin in various media is illustrated in Fig. 20. Extreme positions on the s_{20}-line are marked by $HgCl_2$ and $Co(NH_3)_6Cl_3$. The low sedi-

mentation velocity in the $HgCl_2$ solution is obviously due to the slight dissociation of the salt, and hence its inability to depress the charge effects. The influence of potassium ferrocyanide and hexammine cobaltichloride is quite analogous to that described in the case of gum. The action of uni-univalent salts is intermediate.

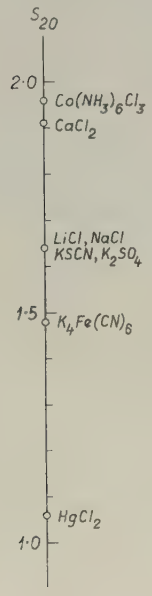

Fig. 20.

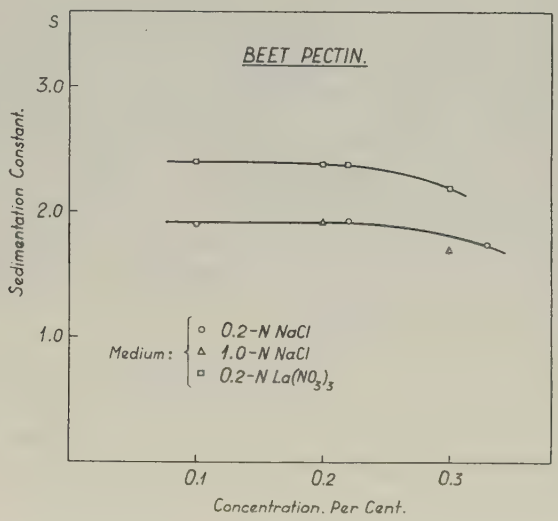

Fig. 21.

Fig. 20.—s_{20} for commercial apple pectin at a concentration of 0·15 per cent in solutions containing various kinds of electrolytes at the concentration 0·05 N.

Fig. 21.—s_{20} for beet pectin as a function of the pectin concentration and the nature of the electrolytic medium.

The addition of lanthanum ions (La^{+++}) to solutions of the type pectins brought about flocculation in the case of apple pectin (high number of free carboxyl groups), and an aggregation to somewhat larger particles in the case of citrus and beet pectin, to judge from the increased sedimentation velocity (Fig. 21). The addition of small amounts of a thorium nitrate solution (Th^{++++}) brought about flocculation in all the pectin solutions.

To avoid complications, media containing uni-univalent salts were used whenever possible, for the determinations of molecular weights (cf. Table 22).

DIFFUSION MEASUREMENTS.

METHOD.

The diffusion of the polyuronides was studied by means of the method worked out by Lamm (88). The construction of the diffusion cell used in the case of aqueous solutions makes possible the formation of a very plain and sharp boundary between solvent and solution. The course of the diffusion is followed optically by means of the scale method described briefly on page 53. By plotting the scale line displacement Z against the scale line position x, a curve is obtained, which in the case of a monodisperse, ideal diffusing substance conforms to the equation

$$\frac{\partial n}{\partial x} = \frac{n_1 - n_0}{2\sqrt{\pi D t}} \cdot e^{-\frac{x^2}{4Dt}}, \tag{22}$$

worked out by Wiener (93) on the basis of the fundamental observations made by Stefan (94) on the lens action of a solution exhibiting a concentration gradient.

n_0 and n_1 are the refractive indices of solvent and solution respectively, D the diffussion constant, and t the time elapsed from the start of the diffusion. $x = 0$ corresponds to the position of the boundary at zero time.

From this basic equation a number of formulas may be obtained for the calculation of D (Lamm, 88). Two of them may be given here. Denoting the area between the curve and the x-axis, i. e.

$$\int_{n_0}^{n_1} \frac{\partial n}{\partial x} dx = n_1 - n_0 \tag{23}$$

by A, and the maximum ordinate or height of the curve by H, we obtain

$$D = D_A = \frac{1}{4\pi t} \cdot \frac{A^2}{H^2}, \tag{24}$$

where the index A indicates calculation by means of the area.

Eq. (22) may also be looked upon as an ideal (Gaussian) distribution function and given the form

$$\frac{\partial n}{\partial x} = \frac{A}{\sigma \sqrt{2\pi}} \cdot e^{-\frac{x^2}{2\sigma^2}}, \tag{25}$$

where σ is the standard deviation. Comparing Eqs. (22) and (25) we find

$$D = D_m = \frac{\sigma^2}{2t}, \tag{26}$$

the index m stresses the fact that the value of σ is usually calculated from the first and the second moment of the curve about its axis.

SIGNIFICANCE OF THE DIFFUSION CONSTANT IN THE CASE OF POLYDISPERSITY.

In the case of a polydisperse system, the values of D calculated by means of Eqs. (24) and (26) are to be regarded as averages of the diffusion constants D_i belonging to particles of varying size and concentration c_i. Using formula (24) we obtain for the average the expression

$$D_A = \left(\frac{\Sigma c_i}{\sum \frac{c_i}{D_i}} \right)^2, \tag{27}$$

[Quensel (95), Gralén (96)]. Formula (26), on the other hand, gives an weight average,

$$D_m = \frac{\Sigma c_i D_i}{\Sigma c_i}. \tag{28}$$

For a detailed discussion of these averages cf. Gralén (96).

Deviations from ideal diffusion, due to polydispersity, intermolecular forces, charge effects etc. will, perhaps, be most clearly evident if we plot the experimental curves referring to different times of diffusion and the ideal curve in one diagram and in what is called normal co-ordinates ('normal analysis', Lamm, 88). The calculation of such co-ordinates is based on data about the area and the standard deviation of the experimental curves in such a way as to eliminate time as a parameter. The curves thus obtained are called *normal curve* and

normal ideal curve respectively. As was shown by Lamm polydispersity reveals itself in the fact that the ordinates of the normal curve in the neighbourhood of $x = 0$ are higher than those of the normal ideal curve, provided the diffusion of the particles of any size proceeds in an ideal manner.

From considerations connected with this fact Gralén (96) showed that the ratio D_m/D_A for a polydisperse substance will, theoretically, always be greater than unity, and proposed that it should be taken as a measure of polydispersity, particularly in the case of chain shaped molecules. It should be borne in mind, however, that the justification of this proposal rests upon the assumption of independent diffusion of particles of varying size. It is clear that the action of intermolecular forces, charges etc., may strongly influence the shape of the diffusion curve and hence the value of D_m/D_A. This was shown by Gralén himself in the case of cellulose in cuprammonium (89).

The diffusion constant will be given below in units of 10^{-7} cm^2/sec. All values are converted to pure water at 20° C as a medium.

CHARGE EFFECTS.

In some very interesting papers on the diffusion of colloidal electrolytes such as starch, gum arabic and dyestuffs, Bruins (97) and Hartley and Robinson (98) have shown the marked influence of the charges on the diffussion velocity. Thus it was shown by Bruins that a preparation of gum arabic had a diffusion constant equal to 25 when diffusing in pure water while, after addition of one milliequivalent of a salt per litre of solution, values of the magnitude 3—7 were obtained, depending on the valency of the ions and whether the ions of high valency were positively or negatively charged.

The experimental technique used either by Bruins or by Hartley and Robinson for the determination of the diffusion constant was such, however, as to give no details about the course of the diffusion. The scale method has the great merit of giving a very detailed picture of the variation of the concentration gradient at any desired moment, and, if wanted, the variation of the concentration itself may be easily pictured by integrating the diffusion curve. Because of these facts some details may be given here about the diffusion of gum arabic and pectic substances in pure water as elucidated by the

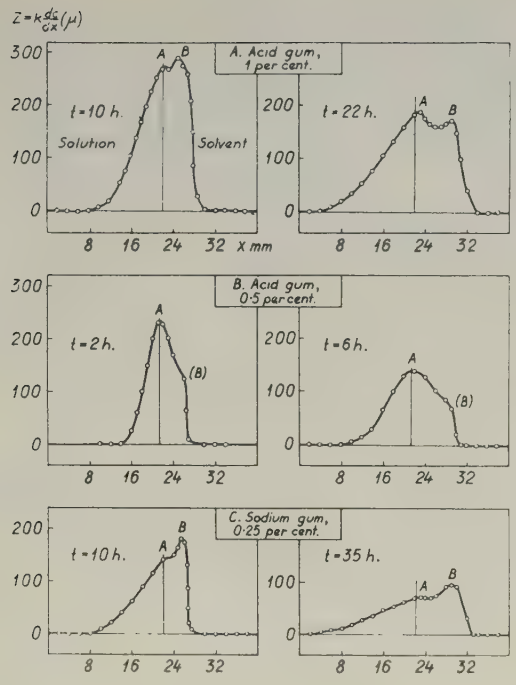

Fig. 22.—Diffusion curves for gum arabic in pure water.

scale method. It might be added that the gum investigated by Bruins had an ash content of no less than 3 per cent. In fact, from measurements of the freezing point depression Bruins concluded that only an insignificantly small amount of the ash constituents was to be regarded as free. This might imply, on the other hand, that a considerable part of the carboxyl groups were occupied in salt formation, and hence the number of free charges was smaller than in a purified substance.

The diffusion of gum arabic is illustrated in Fig. 22. The shape of the curves strongly deviates from the one commonly obtained with uncharged substances. The curves are very steep towards the solvent, in several cases indicating an almost discontinuous transition in concentration. This 'front' or 'border' is largely maintained during the diffusion process and moves under the influence of the diffusion potential set up in consequence of the great difference in the mobility of the gum particles and that of the compensating ions. The rapid movement of the front layer brings about in most cases a division of the substance into two differentiated concentration ranges with

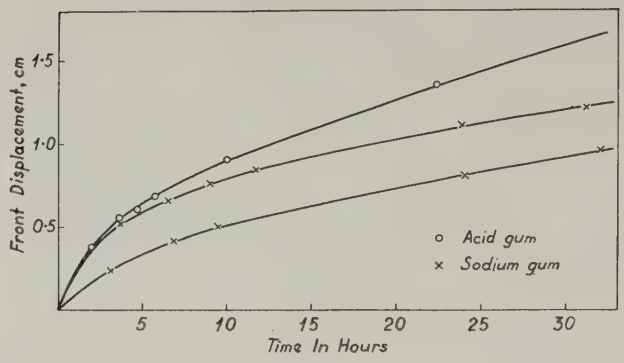

Fig. 23.—Displacement of the 'diffusion front' of gum arabic with time.

maxima A and B respectively. The position of A corresponds to that of the original boundary and does not noticeably change with time, while that of B is determined by the moving front.

From curves of the type shown in Fig. 22 A, C, an approximate value for the velocity of the moving front may be calculated, taking B as an index. In Fig. 23 the position of B is thus plotted against time, the origin denoting the position of the original boundary between solvent and solution. Some values of the front velocity at different times have been calculated from the slope of the curves, Table 26. As may be seen the velocity decreases from a highest value at the start to an almost constant value after about five to ten hours.

It is interesting to compare this velocity with the sedimentation velocity at the same concentration of gum. The average of seven determinations of the sedimentation velocity in 0·2 N-NaCl at a concentration of 0·5 per cent was found to be $9{\cdot}4 \cdot 10^{-5}$ cm/sec. at a rotor

Table 26. — Velocity of the Moving Front.

Time from start, hours	Velocity, (cm/sec) · 10^5	
	Acid gum	Sodium gum
1	4·4	4·4
2	3·8	3·8
3	2·8	2·3
10—20	1·0	0·6

speed equal to 40 000 R.P.M. The velocity in pure water under the same conditions was $5{\cdot}3 \cdot 10^{-5}$ cm/sec., as an average of four determinations. The difference is equal to $4{\cdot}1 \cdot 10^{-5}$ cm/sec., a value quite comparable to that calculated as the velocity of the diffusion front at the beginning of the diffusion process.

At a rotor speed equal to 30 000 R.P.M. the sedimentation velocity of the gum would be $4{\cdot}3 \cdot 10^{-5}$ cm/sec. at a concentration of 0·5 per cent, provided the charge effects were eliminated (cf. Table 22, No. 15; the actual sedimentation velocity at any rotor speed may be calculated from the s_{20}-values). In an experiment at this speed and with pure water as the medium the substance had not come off the meniscus even after 4 hours' run, being distributed, however, in a certain way over the whole cell. In this case the forces acting at the actual boundary obviously balance each other.

The reduction of the sedimentation velocity in pure water may thus be looked upon as being due partly or perhaps mainly to the very strong diffusion potential set up at the boundary (cf. Lamm, 92, p. 7 ff.). This would in reality mean that the sedimentation of the particles within the bulk of the solution would proceed with a velocity differing from that at the actual boundary. As was pointed out above (p. 62) this is in accordance with the shape of the sedimentation curves.

It should be emphasized that the characteristic features of the diffusion of gum arbic pictured above have returned more or less markedly in each of about twenty experiments, and are therefore not to be referred to accidental causes such as convection currents or the like.

The diffusion of the pectins offers a different picture. In this case the curves are rather symmetric or, unlike those for gum arabic, less steep towards the solvent than towards the solution (Fig. 24, *A*, *B*). Curves of the gum type were obtained only in a few experiments at low concentrations (Fig. 24, *C*).

It seems as if the difference in behaviour of the two kinds of polyuronides on diffusion ought to be ascribed to a difference in particle interaction. There is hardly any doubt that the gum particles can move more freely than the pectin particles at equal concentration. This is a result of differences in the shape of the particles and in the 'density' of cohesive centra, i. e. in the first place carboxyl groups. Because of these facts a surface layer of the gum in the diffusion

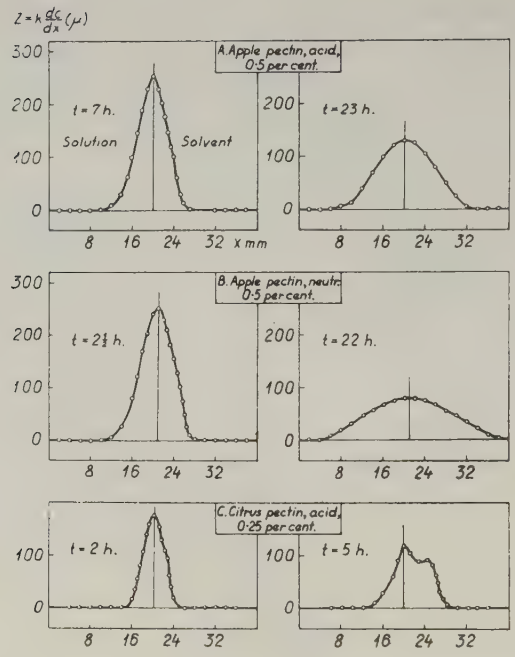
A. Apple pectin, acid, 0·5 per cent.
t = 7 h.
Solution
Solvent
t = 23 h.
B. Apple pectin, neutr. 0·5 per cent.
t = 2½ h.
t = 22 h.
C. Citrus pectin, acid, 0·25 per cent.
t = 2 h.
t = 5 h.
x mm

Fig. 24.—Diffusion curves for pectins in pure water.

column, subjected to a strong diffusion potential, may partly separate from the rest. In the case of pectins the tendency to separation is balanced by the intermolecular forces, except, perhaps, in very low concentrations.

Notwithstanding this fact there are reasons to believe that the effect of the diffusion potential upon the sedimentation of the pectin particles is analogous to that described in the case of gum. The sedimentation diagrams exhibit the characteristic 'diffusion front' where we might suppose the strong diffusion tendency to be maintained during sedimentation (cf. Fig. 17).

In Table 27 some values of the diffusion constant are recorded. By taking curves from early exposures, calculations by means of Eqs. (24) and (26) were also made possible in the case of gum arabic. It may be emphasized that the figures are intended to give orders of magnitude only, the variations in D being rather large owing to the extremely large influence upon the diffusion velocity of traces of electrolytic impurities. In any case, the effect of the diffusion potential on the diffusion velocity of both pectins and gum arabic is enor-

TABLE 27. — DIFFUSION IN PURE WATER.

Conc.: 0·5 per cent. — Temp.: 20° C.

Diffusion time, hours	Apple pectin				Citrus pectin		Beet pectin		Gum arabic	
	Acidic		Neutr.							
	D_m	D_A	D_m	D_A	D_m	D_A	D_m	D_A	D_m	D_A
1	—	—	—	—	—	—	32	31	—	—
2	42	44	39	41	24	29	—	—	44	48
3	—	—	—	—	23	27	28	26	40	42
4	40	43	38	40	21	24	26	25	35	36

mous, the values of the diffusion constants being some twenty times larger than those obtained from the measurements in salt solutions described below. The values calculated for gum arabic are considerably larger than those recorded by Bruins (97).

The marked reduction of the diffusion potential in an electrolytic medium is demonstrated by the data given in Table 28 and by Fig. 25. They refer to the diffusion of gum arabic at a concentration of 0·5 per cent in a medium made up of water containing one milliequivalent of $Co(NH_3)_6Cl_3$, NaCl and $K_4Fe(CN)_6$ respectively per litre. As may be seen, the antagonistic valency effect, emphasized by Bruins, manifests itself clearly both in the values of the diffusion constant and in the shape of the curves. The multivalent ions with negative

TABLE 28. — DIFFUSION OF GUM ARABIC.

Conc.: 0·5 per cent.
Temp.: 20° C.

Diffusion time, hours	Medium: Water containing 1 milliequivalent/l. of					
	$Co(NH_3)_6Cl_3$		NaCl		$K_4Fe(CN)_6$	
	D_m	D_A	D_m	D_A	D_m	D_A
18	3·3	3·0	7·0	5·1	9·2	6·0
30	3·2	2·9	6·8	5·0	7·8	5·3
42	3·2	3·0	6·2	4·9	7·4	4·9

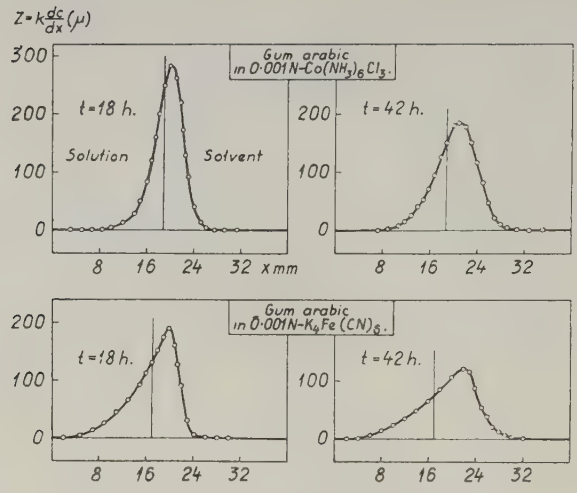

Fig. 25.—Diffusion of gum arabic in dilute electrolytic media. Concentration of gum: 0·5 per cent.

charge are not so effective in depressing the diffusion potential as are those with positive charge, while the univalent ions are intermediate.

The position of the maximum of the diffusion curves is displaced owing to the remaining diffusion potential. After a diffusion time of 18 hours the displacement in the media mentioned above amounts to respectively 1·1, 2·3 and 2·8 mm, after 42 hours the figures are 1·9, 3·7 and 4·8 mm.

TABLE 29. — DIFFUSION OF GUM ARABIC.

Conc.: 0·5 per cent.
Temp.: 20° C.

Diffusion time, hours	Medium: Water containing 5 milliequivalents/l. of					
	$Co(NH_3)_6Cl_3$		NaCl		$K_4Fe(CN)_6$	
	D_m	D_A	D_m	D_A	D_m	D_A
24	2·9	2·5	3·2	2·6	4·0	2·8
43	2·8	2·4	2·8	2·6	3·2	2·4
67	2·6	2·3	-	—	2·5	2·0

At a concentration of salts equal to 5 milliequivalents per litre, practically no displacement of the position of the maximum could be noted in any of the media. The diffusion velocity was further reduced, though not to the same degree in the different media. The values of the diffusion constant (Table 29) approximates to those obtained in a medium containing excess of electrolyte.

Similar effects were noted in the case of pectins, with the exception that the percentage reduction of the diffusion velocity at low concentrations of the salts was not so large as in the case of gum arabic.

Hartley and Robinson (98) in discussing the diffusion velocity of colloidal electrolytes make use of the extended Nernst formula published by Haskell (99),

$$D = \frac{RT}{F} \cdot 10^{-7} \left(\frac{1}{n_+} + \frac{1}{n_-} \right) \frac{uv}{u+v}. \qquad (29)$$

Here n_+ and n_- are the valency, u and v the mobility of cation and anion respectively, and F the Faraday constant. $\frac{RT}{F}$ has the value $2{\cdot}524 \cdot 10^5$ at 20° C.

It might be of a certain interest to see how far the values of D calculated by means of this formula are compatible with those actually determined in the case of the polyuronides. For the acid and neutralized substances dealt with here $n_+ = 1$ and n_- is probably large enough to make the valency factor approximately equal to unity. At 20° C and infinite dilution $u = 337 \cdot 10^{-5}$ cm²/sec.volt for the hydrogen ion and $47 \cdot 10^{-5}$ cm²/sec.volt for the sodium ion. With given values of v the following round values of D are thus obtained.

For acid substances,

v	5	10	15	20	25	30 (cm²/sec. volt) $\cdot 10^{-5}$
D	12	25	36	48	59	70 (cm²/sec.) $\cdot 10^{-7}$.

For sodium salts

v	5	10	15	20	25	30	40 (cm²/sec. volt) $\cdot 10^{-5}$
D	11	21	29	35	41	46	55 (cm²/sec.) $\cdot 10^{-7}$.

On examining the figures given in Table 27 for diffusion in pure water, it is seen that they would require values for v of the magnitude

10—20 in the case of acid substances and 20—25 in the case of neutralized ones, if Eq. (29) applies. These values of v, however, are quite compatible with the electrophoretically determined mobilities u_k given in Tables 20—21 for low ionic strengths. It will thus seem as if the diffusion of the polyuronides in pure water proceeds with good approximation in accordance with the principles underlying Eq. (29).

DIFFUSION IN MEDIA CONTAINING EXCESS OF ELECTROLYTE.

As was shown by Lamm (92) the diffusion of a colloidal electrolyte is affected by the charges even in the presence of excess of electrolyte, and the decisive factors are again mainly those discussed in the case of sedimentation (p. 63). It might, therefore, be concluded from the results of the sedimentation experiments with the polyuronides that the charge effects will be practically eliminated in a medium such as 0·2 N-NaCl. That this does in fact occur might be inferred from the figures given in Table 30 for apple pectin and gum arabic.

Table 30. — Dependence of the Diffusion Velocity upon Concentration of NaCl.

Concentration of the polyuronides: 0·5 per cent.

Diffusion time, hours	Apple pectin in				Gum arabic in			
	0·2 N-NaCl		1 N-NaCl		0·2 N-NaCl		0·5 N-NaCl	
	D_m	D_A	D_m	D_A	D_m	D_A	D_m	D_A
24	2·1	1·7	2·0	1·7	2·6	2·5	2·4	2·3
41	1·9	1·6	2·1	1·7	2·4	2·2	2·5	2·2
66	1·9	1·6	1·8	1·6	2·3	2·2	2·4	2·3

In Tables 31—32 data have been collected showing the dependence of the diffusion velocity upon the concentration of the substance and upon the acidity of the medium.

It can be generally stated that the variation of the diffusion velocity with concentration is astonishingly small as compared to the variation of the sedimentation velocity (Table 22). In the case of gum arabic

Table 31. — Dependence of the Diffusion Velocity of the Polyuronides upon Concentration.

Pectins and gum arabic in 0·2 N-NaCl, sodium alginate in 0·2 N-Na_2NO_3.

Concentration, per cent	Apple pectin		Citrus pectin		Beet pectin		Sodium alginate		Gum arabic	
	D_m	D_A	D_m	D_A	D_m	D_A	D_m	D_A	D_m	D_A
1·00	1·9	1·5	2·0	1·7	—	—	—	—	2·4	2·1
0·75	2·0	1·5	—	—	—	—	3·5	3·4	—	—
0·50	2·0	1·6	1·9	1·6	2·2	2·0	3·2	3·4	2·4	2·2
0·25	2·1	1·8	1·8	1·7	2·0	1·8	3·1	3·0	2·3	2·2
0·10	2·0	1·9	1·7	1·7	1·9	1·8	—	—	2·3	2·2

there is, indeed, no clear variation of the diffusion velocity within the range 1—0·1 per cent, while in the case of the pectins the evidence obtained from D_m and D_A are conflicting. These facts indicate that the frictional forces acting in sedimentation and diffusion are not quite equivalent in the case of these substances and that they are not changed to the same degree by changes in concentration. Similar differences were also found for Helix haemocyanin by Svedberg (100), for various other proteins by Tiselius and Gross (101) and for polymeric ω-hydroxydecanoic acid by Kraemer and Lansing (102). In the case of Helix haemocyanin and polymeric ω-hydroxydecanoic acid it was shown, however, that the frictional constant in sedimentation and diffusion became equal at zero concentration.

The values of D_m and D_A differ to a greater extent the higher the concentration. At the lowest concentratrations there is only a slight difference between the two values (Table 31). This fact is also illustrated in Fig. 26, where the diffusion curves for the various polyuronides are plotted in normal co-ordinates together with the normal ideal curve. At a concentration of 0·5 per cent the maximum ordinate of the normal curves for the pectins especially, is considerably higher than that of the ideal curve. At a concentration equal to 0·1 per cent the difference in height is largely reduced. Similarly the obliquity of the normal curves usually becomes less marked as the concentration decreases. These changes in the shape of the curves are probably mainly due to variations in the strength of the intermolecular forces (for the possible influence of a variation in the refractive index in-

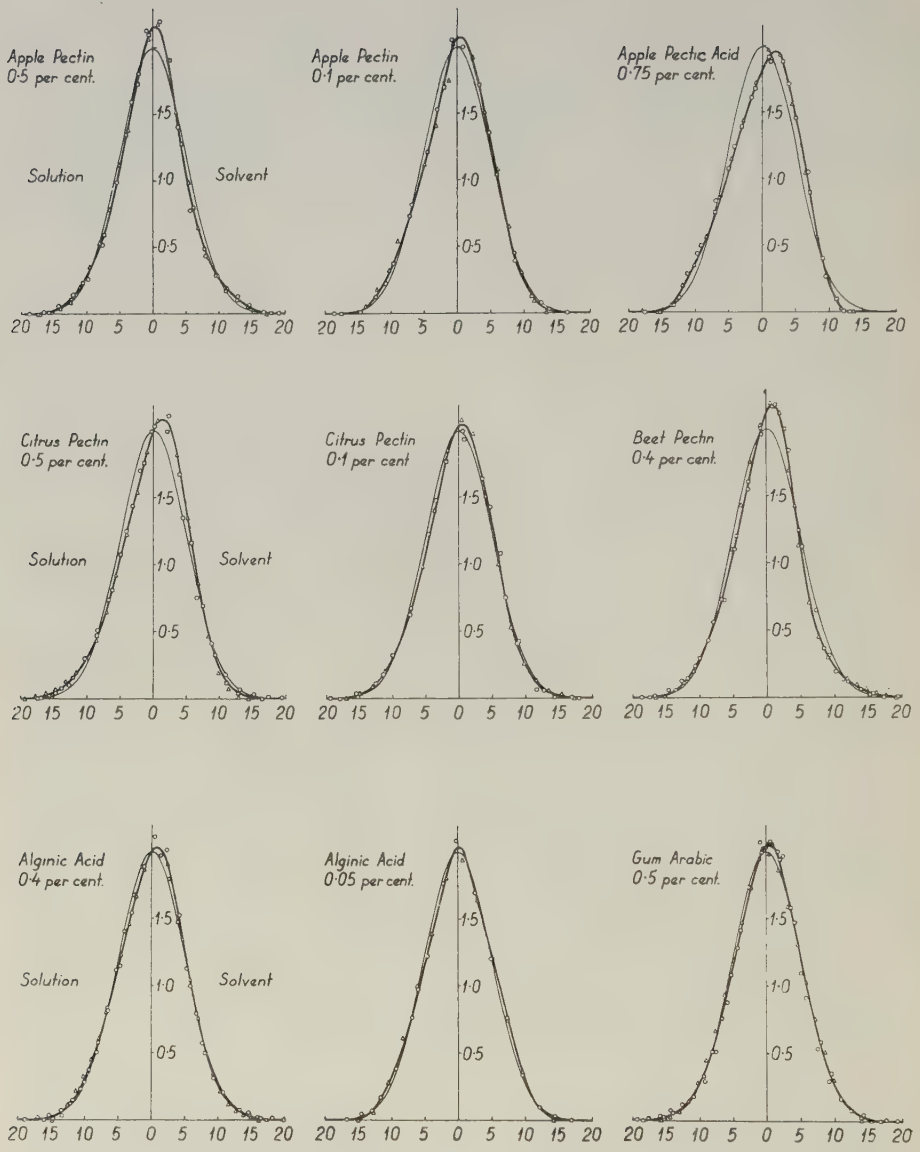

Fig. 26.—Normal diffusion curves for the various polyuronides in 0·2 N salt solutions. The faintly drawn curve is the normal ideal curve. Circles, squares and triangles represent experimentally determined co-ordinates, obtained from exposures taken, on an average, 10, 25, 50 or 25, 50, 80 hours after the beginning of the diffusion process, depending on the concentration of the substance.

crement cf. p. 100). It is in fact significant that the shape of the curves referring to the pectins at very low concentrations more closely resembles that of the curves obtained with sodium pectate or sodium alginate in 0·2 N-Na_2CO_3, even at a concentration of 0·5 per cent or higher. Both dilution and alkaline media bring about a decrease in the magnitude of the intermolecular forces, a fact that is evident most clearly perhaps from the strong decrease in viscosity brought about by either means.

Obviously no accurate conclusions can be drawn from the ratio D_m/D_A as to the degree of polydispersity under such circumstances, except perhaps in the case of the lowest concentrations (cf., however, the values of D_m/D_A given in Table 33 with the evidence obtained from sedimentation equilibrium, Table 49).

TABLE 32. — DEPENDENCE OF THE DIFFUSION VELOCITY UPON pH.

Concentration of the polyuronides: 0·5 per cent. Medium: McIlvaine's standards.[1]

pH	Apple pectin $Cx = 21{\cdot}4$[2]		Citrus pectin $Cx = 5{\cdot}8$		Beet pectin $Cx = 7{\cdot}6$		Gum arabic $Cx = 8{\cdot}3$	
	D_m	D_A	D_m	D_A	D_m	D_A	D_m	D_A
1·1	1·4	1·5	—	—	—	—	2·2	2·1
2·4	1·7	1·5	1·7	1·6	—	—	—	—
3·0	2·2	1·7	—	—	2·3	2·1	2·5	2·3
3·7	2·3	1·9	1·8	1·6	—	—	—	—
4·9	2·4	1·8	—	—	—	—	—	—
6·9	2·5	2·1	1·8	1·6	2·8	2·5	2·7	2·5

From the data collected in Table 32 (concentration of 0·5 per cent) it may be seen that the diffusion velocity of the type substances is markedly dependent upon the acidity of the medium, citrus pectin forming presumably an exception. Again, we might suppose the action of cohesive forces, located to or regulated by the carboxyl groups especially, to be responsible for this dependence. This supposition is confirmed by a comparison of the degree of variation of D with the

[1] Mixtures of 0·2 M-Na_2HPO_4 and 0·1 M-citric acid. — For the measurement at pH = 1·1 0·2 M-NaCl was used + the proper amount of HCl.

[2] $Cx = 10^4$ times the number of free carboxyl groups per gram of the substance.

number of free carboxyl groups per gram of the substance (Cx in Table 32).

Whether the action of the cohesive forces brings about the formation of aggregates which may be defined as kinetic units is not clear. In the case of apple pectin, for instance, the sedimentation velocity in acid media does not differ from that in neutral media to a degree corresponding to the difference in diffusion velocity in the two kinds of media. In the case of gum arabic, again, both sedimentation velocity and diffusion velocity changes in a way that may be interpreted as indicating the formation of larger particles in acid media.

TABLE 33. — DIFFUSION CONSTANTS OF THE POLYURONIDES. — 20° C.

No.	Preparation[1]	Medium	Conc. per cent	D_m	$D_{.1}$	$D_m/D_{.1}$
1	Free apple pectin	0·2 N-NaCl	0·16	0·90	0·79	1·14
2	Apple pectin acc. to v. Fellenberg	» »	0·14	2·2	2·0	1·10
3	Commercial apple pectin, acidic	» »	0·10	1·9	1·8	1·06
4	Do., neutralized with NaOH	» » , pH adjusted to 7	0·10	2·1	2·0	1·05
5	Pectic acid from comm. apple pectin	0·2 N-Na_2CO_3	0·25	1·9	2·1	0·90
6	Free citrus pectin	0·2 N-NaCl	0·06	0·63	0·60	1·05
7	Extracted citrus pectin, acidic	» »	0·10	1·5	1·4	1·07
8	Do., neutralized with NaOH	» » , pH adjusted to 7	0·10	1·7	1·7	1·00
9	Beet pectin	0·2 N-NaCl	0·10	1·9	1·8	1·06
10	Red currant pectin	» »	0·10	2·9	2·7	1·07
11	Carrot pectin	» »	0·20	2·3	1·9	1·21
12	Flax pectin (A)	0·2 N-$(COONH_4)_2$	0·25	2·1	1·6	1·31
13	Alginic acid	0·2 N-Na_2CO_3	0·25	3·1	3·0	1·03
14	Do., commercial	» »	0·10	1·9	1·7	1·12
15	Gum arabic, acidic	0·2 N-NaCl	0·25	2·3	2·2	1·05
16	Do.	» » + HCl, pH 1·1	0·25	2·2	2·1	1·05
17	Do.	0·1 N-$Th(NO_3)_4$	0·25	2·1	2·2	0·95
18	Sodium gum	0·2 N-NaCl+NaOH, pH 7	0·25	2·7	2·5	1·08

[1] Cf. p. 16.

Values of the diffusion constant for the various polyuronides are collected in Table 33. They refer to a concentration chosen in order to reduce the action of intermolecular forces as far as possible, and yet permit the determination of the diffusion constant (i. e. plotting of the diffusion curve) with sufficient accuracy. They will be used for calculating the molecular weights.

THE PARTIAL SPECIFIC VOLUME.

The calculation of molecular weights by means of formula (14), p. 54, requires a knowledge of the partial specific volume V of the substance in question. Values of this quantity do not seem to have been published in the case of the polyuronides, and it should, therefore, not be out of place here to give some details of determinations of V for the type substances dealt with above.

The values given below were determined by means of a pycnometer holding about 55 ml. The partial specific volume was calculated according to the formula

$$V = v\left(1 - \frac{100 - p}{m} \cdot \frac{dm}{dp}\right), \tag{30}$$

(cf. Kraemer in UC, p. 57 ff.) where v is specific volume of the solution, m weight of the pycnometer content, and p concentration of the substance in weight per cent.

The pycnometer with content, of weight M, was weighed against a tare having a volume approximately equal to that of the pycnometer and containing water to the weight $T \approx M$. The weighings thus gave, with the

test solution, $M = T + \Delta m$,

and with pure water, $M_0 = T + \Delta m_0$,

whence $M - M_0 = m - m_0 = \Delta m - \Delta m_0$, or

$$m = k + \Delta m,$$

where $k = m_0 - \Delta m_0$ is a constant referring to pure water.

The various solutions were prepared from a stock solution, purified by dialysis as described on page 16. The concentration of this solution was determined by evaporating 2 à 3 grams of it at 105° C over night in small weighing bottles. The average of three determinations was recorded as 'concentration'. Deviations from the mean, amounting to $\pm$ 0·3 per cent, were noted at concentrations of the magnitude 2 per cent. A series of test solutions was prepared by

Table 34. — Partial Specific Volume of Apple Pectin at pH 7.

p	Δm	m	v	V
1·349	0·5964	55·6714	0·99045	0·6026
0·899	0·4958	55·5708	0·99224	0·6012
0·599	0·4292	55·5042	0·99343	0·6003
0·399	0·3850	55·4600	0·99422	0·5996
0·200	0·3412	55·4162	0·99501	0·5990
Zero	0·2971	55·3721	0·99580	0·5984

successive dilutions with the proper buffer, the quantities always being determined by weighing. The determination of V for apple pectin (type substance) is exemplified by the data given in Table 34. A phosphate buffer with pH 7 was used as a medium. The values refer to a temperature of $20 \pm 0{\cdot}01^\circ$ C. Fig. 27 shows that the function $\Delta m = f(p)$ is strictly linear, giving $dm/dp = 0{\cdot}221$ in this particular case. A slight decrease in V with concentration was found throughout these measurements. In Table 35 the variation of V with pH is illustrated. The values of V are averages within the concentration range 1—0 per cent, the maximum deviation from the mean being of the magnitude 0·3 per cent.

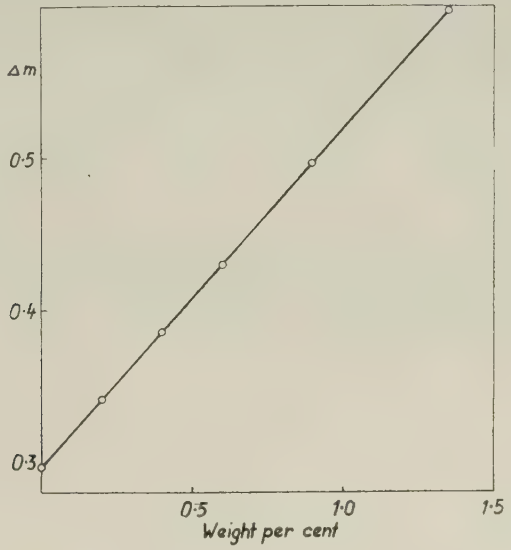

Fig. 27.—Variation of Δm with concentration.

TABLE 35. — VARIATION OF THE PARTIAL SPECIFIC VOLUME WITH pH. — 20° C.

Medium	pH	Apple pectin $Cx = 21{\cdot}4$[1]	Citrus pectin $Cx = 15{\cdot}5$	Citrus pectin $Cx = 5{\cdot}8$	Beet pectin $Cx = 7{\cdot}6$	Gum Arabic $Cx = 8{\cdot}3$	Alginic acid $Cx = 52$	Methyl cellulose[3] $Cx = 0$
0·10 N-HCl . . .	1	0·569	0·585	0·603	—	—		0·708
0·01 N-HCl . . .	2	—	0·587	—	0·601	0·612		—
McIlvaine's standards[2] . . .	3	0·570	0·586	0·604	—	—		0·707
	3·6	—	0·589	—	—	—		—
	4	—	—	—	0·601	0·619		—
	4·4	—	0·595	—	—	—		—
	5	0·579	—	—	—	—		0·707
	5·5	—	0·604	—	—	—		—
	7	0·601	0·612	0·619	0·619	0·622		0·709
0·1 N-NaOH . . .	13	—	—	—	—	—	0·605	0·706

The figures show that V decreases with increasing hydrogen ion concentration, and the more so the larger the number (Cx) of free carboxyl groups per gram of the substance. Methyl cellulose, which was included for the purpose of comparison, exhibits no variation in V with pH. Obviously the decrease is connected with the suppression of the dissociation of the carboxyl groups. As was pointed out above, in connection with the discussion of the viscosity changes with pH (p. 31), it is probable that this suppression of the dissociation is followed by changes in the degree of hydration of the particles. Such changes might possibly contribute to the variation of V if we may assume the hydration water and the free water to differ markedly in density.

[1] $Cx = 10^4$ times the number of free carboxyl groups per gram of the substance.

[2] Mixtures of 0.2 M-Na_2HPO_4 and 0·1 M-citric acid.

[3] Methoxyl content, 24 per cent.

MOLECULAR CONSTANTS FROM SEDIMENTATION AND DIFFUSION.

From the data collected on the foregoing pages we may estimate several molecular constants by using formulas which are in fact strictly applicable only to monodisperse substances.

MOLECULAR OR PARTICLE WEIGHT.

Values of the molecular or particle weight M of the various polyuronides are given in Table 36. They have been calculated by means of formula (14), p. 54,

$$M = \frac{RTs}{D(1 - V\varrho)},$$

using the value of s at zero concentration given in Table 22 and the mean of D_m and D_A given in Table 33. These values refer to pure water at 20° C, so that $\varrho = 1{\cdot}00$.

The validity of formula (14) rests upon the assumption that the molar frictional constant in sedimentation f_s is equal to that in diffusion f_D, where

$$f_s = \frac{M(1 - V\varrho)}{s} \text{ and } f_D = \frac{RT}{D}. \qquad (31)$$

We can, however, never expect f_s to be identical with f_D in the case of a polydisperse system. As was shown on p. 56, in the case of negligible diffusion the sedimentation constant at zero concentration will refer to a single particle magnitude, viz. that which occurs most frequently in the particle size distribution. The values of D (D_m or D_A), on the other hand, is determined by diffusion of all kinds of particles present, varying in magnitude and in the value of the molar frictional constant, the whole diffusion curve being taken into consideration. It was also shown above (p. 79) that since the sedimentation velocity and the diffusion velocity depend to different degrees upon concentration, f_s and f_D will differ to some extent.

Nevertheless, the values of M given in Table 36 may be taken, in most cases, as rather good averages, as will be evidenced by their compatibility with those obtained from the sedimentation equilibrium measurements to be described below. This means that the average values of f_D at low concentrations determined experimentally will agree rather well with the value of f_s belonging to the particles of highest frequency in the probably rather symmetrical particle size distribution of the polyuronides (cf. p. 56).

For a discussion of the various possible molecular averages which can be obtained by applying formula (14) to a polydisperse substance, cf. Jullander (103).

TABLE 36. — MOLECULAR CONSTANTS OF THE POLYURONIDES.

No.	Preparation	Medium used in measurement	s_{20}	D_{20}	$(1-V\varrho)_{20}$	M	f/f_0	l/d	l in Ångström units	d
1	Free apple pectin . . .	0·2 N-NaCl	2·8	0·85	0·40	201 000	6·9	290	3 200	11
2	Apple pectin acc. to v. Fellenberg	» »	2·0	2·1	0·40	58 000	4·2	110	1 100	10
3	Commercial apple pectin, acidic	» »	2·2	1·9	0·42	67 000	4·5	120	1 200	10
4	Do. neutr. with NaOH .	» » , pH 7	2·0	2·1	0·40	58 000	4·2	110	1 100	10
5	Pectic acid from comm. apple pectin	0·2 N-Na_2CO_3	2·0	2·0	0·39	62 000	4·3	110	1 100	10
6	Free citrus pectin . . .	0·2 N-NaCl	4·0	0·62	0·38	414 000	7·4	340	4 500	13
7	Extracted citrus pectin, acidic	» »	2·2	1·5	0·40	89 000	5·1	160	1 600	10
8	Do. neutr. with NaOH .	» » , pH 7	2·0	1·7	0·38	75 000	4·7	140	1 400	10
9	Beet pectin	» »	1·9	1·9	0·39	62 000	4·5	120	1 200	10
10	Red currant pectin . . .	» »	2·0	2·8	0·41	42 000	3·5	70	730	10
11	Carrot pectin	» »	2·0	2·1	0·40	58 000	4·2	110	1 100	10
12	Flax pectin (A)	0.2 N-$(COONH_4)_2$	2·0	1·9	0·40	64 000	4·5	120	1 200	10
13	Alginic acid	0·2 N-Na_2CO_3	2·4	3·0	0·39	50 000	3·1	60	700	12
14	Do., commercial	» »	3·0	2·5	0·39	75 000	3·2	60	800	13
15	Gum arabic acidic . . .	0·2 N-NaCl,	10·7	2·3	0·38	298 000	2·2	25	700	28
16	Do.	» » , pH 1·1	11·5	2·2	0·39	326 000	2·3	28	785	28
17	Sodium gum	» » , pH 7	10·4	2·6	0·38	256 000	2·1	23	635	28

As may be seen from Table 36, the molecular weight of the free pectin from apple juice and lemon albedo is far greater than that of the pectins obtained by boiling the vegatable materials. Boiling, whether in distilled water (No. 2) or in markedly acid medium (pH 2—3 in most extractions) obviously brings about a splitting up of the molecules as they probably exist *in situ* in the plant tissues. The free pectin from red currant has probably suffered from the rather high acidity of its native environment.

The molecular weight of the type pectins (No. 3, 7 and 9) is of special interest from the point of view of their jellying power. As was mentioned above, p. 40, Myers and Baker, and Schneider and Bock consider that the jellying power of the pectins depends mainly upon the degree of polymerization of their supposedly chain shaped molecules, i. e. upon their molecular weight. This view is, in fact, supported by the fact that the citrus pectin has a molecular weight some 30 per cent higher than that of the apple pectin and also, as was shown above, a jellying power considerably higher than that of the apple pectin. On the other hand, beet pectin has a molecular weight comparable to that of the apple pectin but practically no jellying power. The same was found to be the case in flax pectin. The conclusion must be drawn that there are, in addition, differences in composition and perhaps in constitution which may influence the jellying power of pectins. We have already seen (p. 21) that beet pectin contains acetyl groups while no such groups are to be found in apple or citrus pectin. As was emphasized by Hinton (104), the ratio of the number of free (non-esterified) carboxyl groups to the whole number of carboxyl groups in the molecule, and the degree of ionization are other factors, to be taken into account.

The molecular weight of alginic acid is of the same magnitude as that of the pectins obtained by hot extraction. The necessary use of a rather strongly alkaline medium (0·2 N-Na_2CO_3) as a solvent for the acid involves the risk that the substance may be partially destroyed. Both pectins and alginic acid are very sensitive to the action of alkali. In the case of pectic acid (No. 5) it was also noted that the diffusion velocity slowly increased with time (D_{20} in Table 33 refers to early exposures), which probably indicates that the substance is slowly decomposing. No corresponding increase in D was observed, however, in the case of alginic acid. From the value of M for pectic

acid we might also conclude that no serious decomposition takes place during the time needed for measurement.

The molecular weight of gum arabic varies with the acidity of the medium. This fact is in close agreement with the results arrived at by Oakley in his osmotic pressure measurements on gum arabic, commented upon on pp. 14 and 66. The pectins exhibit similar, though not so marked, variations in M with the acidity of the medium. The probable reasons for this fact were discussed on pp. 65 and 81.

THE SHAPE OF THE PARTICLES.

An indication of the shape of the particles constituting the polyuronides in aqueous solution may be gained from the following considerations (Svedberg and Pedersen, UC p. 38 ff.).

According to Stokes's law the molar frictional constant f_0 for a substance made up of spherical, not solvated particles may be written

$$f_0 = 6\pi\eta N\left(\frac{3MV}{4\pi N}\right)^{1/3}. \tag{32}$$

From the values of D and s, on the other hand, we have

$$f = \frac{RT}{D} = \frac{M(1-V\varrho)}{s}. \tag{33}$$

Referring the experimental values to water at 20° C we obtain from Eqs. (32) and (33)

$$f/f_0 = 10^{-8}\left(\frac{1-V\varrho}{D_{20}^2 s_{20} V}\right). \tag{34}$$

If f/f_0 in this equation equals unity, we are thus dealing with a substance made up of spherical, not hydrated particles. In cases where f/f_0 is greater than unity this may be taken as either an indication of hydration or of a particle shape deviating from the spherical, or of both factors. If the particles can not move independent of one another this might, of course, also contribute to the increase in f/f_0.

The particles of the polyuronides in aqueous solution are no doubt hydrated to a certain degree. But we may also assume that they deviate markedly from the spherical owing to their probable chain structure. The high value of f/f_0 recorded in Table 36 for the various polyuronides shows, in fact, that both assumptions may be justified.

Now on the assumption that the particles may be looked upon as oblong ellipsoids of length l and thickness d, and if hydration may be ignored, f/f_0 is related to the ratio $l/d = 1/p$ by means of the formula

$$f/f_0 = \frac{\sqrt{1-p^2}}{p^{\frac{2}{3}} \log \frac{1+\sqrt{1-p^2}}{p}}, \tag{35}$$

deduced by Herzog, Illig and Kudar (105).

Numerical values of l/d for various values of f/f_0 are to be found in tables calculated by Svedberg and Pedersen (UC, p. 41) and by Gralén (89, p. 96). By means of these tables values of l/d for the various polyuronides have been recorded in Table 36.

From the relation between molecular weight, partial specific volume and the assumed ellipsoidal shape of the particles, i. e.

$$\frac{\pi l d^2}{6} = \frac{M V}{N}, \tag{36}$$

(N = the Avogadro constant) and the value of l/d we may finally arrive at the separate values of l and d (Table 36).

TABLE 37.

l/d	K	l/d	K
1	2·50	40	39·4
2	2·58	45	46·5
4	3·08	50	55·3
6	3·84	60	74·6
8	4·80	75	108·4
10	5·93	100	177
12	7·20	125	261
15	9·38	150	358
20	13·7	175	470
25	18·8	200	595
30	24·6	250	885
35	31·2	300	1 227

Now it is clear from what has been said above, that the dimensions calculated in this way may be largely fictitious. In order to decide whether there is any real meaning behind them, it is necessary to make comparisons with results obtained by other methods.

Viscosity measurements, for instance, offers another way of determining l/d. For a dilute suspension of ellipsoidal particles Burgers (106) gives the equation

$$100\,[\eta] = V \cdot K \tag{37}$$

where K is a function of l/d having the numerical values presented in Table 37 (106, p. 153).

The maximum length of the supposed chain shaped molecules of pectins and alginic acid can be calculated from the degree of polymerization (D. P.) and the length of the polymerizing unit, the latter being put equal to that of the glucose unit in cellulose, i. e. 5·15 Ångström units. We thus obtain for pectins

$$l = \frac{5 \cdot 15\,M}{183}, \tag{38}$$

and for alginic acid

$$l = \frac{5 \cdot 15\,M}{175}, \tag{39}$$

(cf. p. 24, footnote)

Finally a value for l may be obtained from measurements of streaming birefringence.

The results obtained by applying these different methods of calculation to the type substances are to be found in Table 38.

Table 38. — Particle Dimensions of the Type Substances.

Preparation	l/d according to		l in Ångström units, according to				d in Å units, acc. to	
	f/f_0	$[\eta]$	f/f_0, M	$[\eta]$, M	D. P.	stream. birefr.	f/f_0, M	stream. birefr.
Apple pectin . . .	120	215	1 200	1 700	1 900	2 630	10	7
Citrus pectin . . .	160	280	1 600	2 400	2 500	3 780	10	7
Beet pectin . . .	120	180	1 200	1 600	1 800	—	10	—
Alginic acid . . .	60	300	700	2 000	1 500	—	12	—
Gum arabic . . .	25	35	700	880	—	1 100	28	23

As was to be expected there are rather large variations in the values obtained by the various methods. The figures for alginic acid especially differ markedly, while those for gum arabic are rather consistent. There is no doubt, however, that we are dealing with particles very elongated in shape. This is especially obvious from the streaming birefringence measurements.[1]

It is particularly interesting to note that the shape of the gum particles, too, strongly deviates from the spherical. Several workers on gum arabic have assumed them to be spherical in shape. Hartley and Robinson (98), and Bruins (97), for instance, on discussing the diffusion of gum arabic made use of the Stoke-Einstein equation,

$$D = \frac{R\,T}{N} \cdot \frac{1}{6\,\pi\,\eta\,r}, \tag{40}$$

where r is the particle radius, for the calculation of D with adopted values of r. This will obviously lead to erroneous results if we are dealing with particles for which l/d is of the magnitude 28.

The thickness d of the particles is of particular interest because this magnitude may be compared with data obtained from X-ray analyses and with the conceptions developed as to the structure of the molecules. Taking the values of d obtained from f/f_0 and M we find, to begin with, that the gum particles have a diameter considerably larger than that of the pectin and algin particles. This is quite in accordance with the structural patterns shown in Figs. 1, 3 and 4.

The thickness of the pectin particles belonging to various kinds of pectins is, furthermore, surprisingly constant, being of the magnitude 10 Å, except for the extraordinarily high molecular free pectins. Adhering to the ellipsoidal model we may thus calculate a cross-sectional area of the

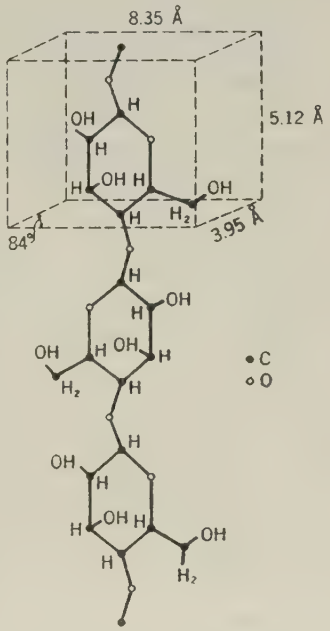

Fig. 28.—Model of the cellulose chain. (Meyer and Lotmar, 107.

[1] The streaming birefringence measurements were performed by Dr. O. Snellman at this Institute.

magnitude 80 Å^2. If we may assume the uronic acid unit to have dimensions comparable to that of the glucose unit in cellulose — and such an assumption seems very reasonable — the cross-sectional area would be about 33 Å^2 (Fig. 28) and we might in consequence assume the pectin particles to be made up of double chains. This assumption, however, would require a maximum length of the particles only half as large as that calculated from M and D. P. (Table 38). Actually the values of l according to f/f_0 and M are about two thirds of the maximum length. Taking into account, in addition, the improbability of maximally extended molecules we are forced to the conclusion that the particles are identical with single chain molecules, acting kinetically as particles of the dimensions calculated.

The dimensions of the alginic acid particles (according to f/f_0 and M) differ so markedly from those of the pectin particles that we cannot preclude the possibility of a micell formation.

THE VISCOSITY-MOLECULAR WEIGHT RELATION.

From what has been said above it may be justifiable to look upon the particles of pectin and alginic acid as 'linear macromolecules', and hence we may assume Staudinger's (58) molecular weight relation

$$\eta_{sp}/c_{gm} = K_m \cdot M \qquad (41)$$

to be valid, provided the charge effects are eliminated (cf. p. 30). In order to test the applicability of Eq. (41) some values of the Staudinger constant K_m were calculated from the figures for $[\eta]$ and M to be

Table 39. — Viscosity-Molecular Weight Relation.

Preparation	$[\eta]$	η_{sp}/c_{gm} [1]	M	$K_m \cdot 10^4$	Medium
Cirus pectin	6·45	118·0	89 000	13·3	0·2 N-NaCl
Commercial apple pectin	3·84	70·3	67 000	10·5	» »
Beet pectin	2·93	53·6	62 000	8·7	» »
Alginic acid	7·72	135·1	50 000	27·0	0·2 N-Na_2CO_3
Do., commercial	10·3	180·2	75 000	24·0	» »

[1] Cf. p. 24, footnote.

found in Tables 9 and 36. As may be seen from Table 39 the values thus obtained differ markedly in the case of pectins. A rough estimation of the average molecular weight of pectins may probably be obtained from viscosity measurements by taking a K_m-value of the magnitude $10 \cdot 10^{-4}$.

The K_m-values for alginic acid are rather consistent, but are about twice as large as those obtained for the pectins.

SEDIMENTATION EQUILIBRIUM.

It is of great value that the ultracentrifuge technique renders it possible to determine molecular weights by means of two essentially different methods. We have already made use of the sedimention velocity combined with diffusion measurements. In this chapter we shall examine the results obtained from measurements of the so called sedimentation equilibrium.

METHOD.

The sedimentation equilibrium is studied by means of an ultracentrifuge of special construction, the number of revolutions not exceeding 18 000 R.P.M. (low speed ultracentrifuge; for details cf. UC). The scale method is used in most cases for observation purposes.

By choosing a suitable number of revolutions a state of equilibrium is finally attained, when the amount of substance sedimenting through unit area at any point of the cell is equal to that diffusing in the opposite direction. After equilibrium has been reached the concentration distribution (c, x) in the cell may be calculated from a (Z, x) diagram obtained as described above (p. 54). The molecular weight is then calculated from the equation

$$M = \frac{2\,R\,T \log\,(c_2/c_1)}{(1 - V\varrho)\,\omega^2\,(x_2^2 - x_1^2)} = \frac{R\,T \cdot dc/dx}{(1 - V\varrho)\,\omega^2\,x\,c_x}, \qquad (42)$$

(UC, p. 304 ff.) where c_1, c_2 and c_x are the concentrations at the points x_1, x_2 and x respectively. The value of dc/dx at any point of the cell is calculated from the scale line displacement Z by means of Eq. (12), p. 53,

$$\frac{dc}{dx} = \frac{Z}{G\,a\,b\,\alpha}.$$

In the case of a monodisperse substance the molecular weight can also be calculated from the formula

$$M = \frac{2\,R\,T\,\log\,(Z_2\,x_1/Z_1\,x_2)}{(1 - V\varrho)\,\omega^2\,(x_2^2 - x_1^2)}, \tag{43}$$

where Z_1 and Z_2 are the scale line displacements at the points x_1 and x_2 respectively.

For a polydisperse solute Eqs. (42) and (43) give values M_i varying with the position x_i in the cell. As was shown by Lansing and Kraemer (108) any such value M_i, obtained by means of Eq. (42), is a weight-average molecular weight at that particular position x_i and concentration c_i in the cell. A weight-average molecular weigt M_w for the entire sample may be obtained by applying the expression

$$M_w = \frac{\Sigma\, c_i\, M_i}{\Sigma\, c_i}, \tag{44}$$

the summation being extended over the length of the cell.

This weight-average M_w should not be confused with the number-average

$$M_n = \frac{\Sigma\, n_i\, M_i}{\Sigma\, n_i} = \frac{\Sigma\, c_i}{\Sigma\, c_i/M_i}, \tag{45}$$

which would, for instance, have been obtained from osmotic molecular weight determinations.

The application of Eq. (43) gives a third average molecular weight M_z, defined by the expression

$$M_z = \frac{\Sigma\, c_i\, M_i^2}{\Sigma\, c_i\, M_i}. \tag{46}$$

This average is neither a number-averge nor a weight-average value. It is given here because of its significance for the estimation of the degree of polydispersity.

For a monodisperse system

$$M_n = M_w = M_z, \tag{47}$$

in the case of polydispersity, however,

$$M_n < M_w < M_z. \tag{48}$$

Lansing and Kraemer (108) showed that the data obtained from measurements of sedimentation equilibrium afford a means of characterizing the degree of polydispersity. Assuming a logarithmic molecular weight distribution, defined by the equation

$$dw = \frac{W}{M_n \beta \sqrt{\pi}} \cdot e^{-y^2} dM, \tag{49}$$

where

$$y = \frac{1}{\beta} \log \frac{M}{M_0},$$

dw the weight of the molecules of a magnitude between M and $M + dM$, and M_0 the magnitude of the molecules of maximum frequency, a value of the 'non-uniformity coefficient' β, i. e. a magnitude defining the spread of the distribution. can be calculated from the equations

$$\frac{M_z}{M_w} = \frac{M_w}{M_n} = e^{1/2 \beta^2} \tag{50}$$

$\beta = 0$ means monodispersity.

THE REFRACTIVE INDEX INCREMENT.

The calculation of M_w by means of Eq. (42) requires a knowledge of the refractive index increment $\alpha = \frac{dn}{dc}$ (p. 96). This magnitude, for the wave-length $\lambda = 436$ mμ used in the measurements, was determined by means of a double prism following the principles described by Kessler in Handbuch der Physik (109). The deviation of the light beam was recorded photographically.

In addition values of α were also determined from calculations based on the properties of the diffusion curves. α may be calculated from the equation

$$\alpha = k \cdot \frac{A}{c}, \tag{51}$$

where k is a constant including factors dependent upon the optical arrangement in the diffusion experiment and upon the choice of coordinates for the diffusion curve, A the area of the curve, and c the concentration.

TABLE 40. — REFRACTIVE INDEX INCREMENT α OF THE POLYURONIDES AT 20° C.

$\lambda = 436$ mμ

Preparation	Medium	pH	Conc. per cent	$\alpha \cdot 10^5$ from measurements by means of	
				double prism	diffusion
Commercial apple pectin	0·2 N-NaCl	2·38	1·00	143	140—148
			0·80	141	
			0·60	142	
			0·40	141	
			0·30	138	
			0·20	137	
		3·00	0·10	136	
		3·40	0·05	—	130—137
	0·2 N-NaCl+ varying amounts of 0·1 N-NaOH	3·58	0·525	144	—
		5·25		139	141
		6·52		135	—
		6·77		129	130
		8·88		127	—
Citrus pectin	McIlvaine's buffer	3·05	1·00	146	—
	»	6·61	»	137	—
	0·2 N-NaCl	3—4	0·10—0·05	—	130—144
Beet pectin	0·2 N-NaCl	3·12	0·44	145	140—150
Pectic acid	0·2 N-Na_2CO_3	10·8	0·596	114	130
Alginic acid	» »		0·513	115	120—130
Gum arabic, acid	0·2 N-NaCl	2·7—3·2	0·50—0·10	—	150—160
Do. neutralized with NaOH	» »	7	0·491	147	160—166
Do.	» »	»	3·775	145	—

From the figures given in Table 40 it might be inferred that the values of α obtained by means of the two methods agree fairly well, and that they differ only slightly for the various kinds of polyuronides.

To judge from the figures for commercial apple pectin, especially, there seems to be a decrease in the increment when concentration becomes smaller than about 0·3 per cent. This fact is also of a certain interest for a special reason. As may be seen from Fig. 26, p. 80, the diffusion curves in practically every case exhibit a more or less marked obliquity. Such an obliquity would necessarily be brought about if α decreased with concentration. Of course, this does not mean that a variable increment is the only possible reason for obliquity.

The increment of the pectin solutions also decreases with increasing pH. Although it is not quite evident from the figures given in Table 40, it is probable that the dependence of α upon pH is connected with the number of free carboxyl groups per gram of the substance in question. This assumption is confirmed by the fact that the increment for alginic and pectic acid in 0·2 N-Na_2CO_3, i. e. for the two substances with the greatest possible number of free carboxyl groups, in a highly alkaline medium, exhibits an exceptionally low value.

The increment for gum arabic seems to be less variable with concentration. Its value in neutral solution is distinctly higher than that for neutralized apple and citrus pectin.

In calculating M_i for the pectins, an average value for α was taken, allowing for the variation of c in the cell.

MOLECULAR WEIGHTS.

Data referring to the sedimentation equilibrium of the type substances at 20° C are given in Tables 41—48.

From the experience gained above (pp. 63, 78) we might presume that the influence of the charges would be practically eliminated in these measurements also by using as solvents electrolytic media of the kind indicated in the tables (cf. Lamm, 92).

Equilibrium was usually reached after about 10—12 days in the case of pectins and alginic acid, and after about 8 days in the case of gum arabic.

Great care had to be taken, particularly in summer, to avoid the action of fungi and bacteria. Several experiments were spoilt by attacks of such organisms, the substance being broken down to fragments of the molecular magnitude 10 000 during the time needed to reach a state of equilibrium.

TABLE 41. — COMMERCIAL APPLE PECTIN, I.

($M_s = 67\,000$)

Medium: 0·2 N-NaCl. $\alpha = 140 \cdot 10^{-5}$. 7 200 R.P.M.

$c = 0{\cdot}50$ p. c.					$c = 0{\cdot}30$ p. c.				
x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
4·73	82	0·276	41 000	57 700	5·42	65	0·153	42 800	39 600
4·78	95	0·305	42 500	68 300	5·47	73	0·172	42 400	38 500
4·83	113	0·339	45 000	78 700	5·52	82	0·193	42 100	38 500
4·88	138	0·379	48 600	81 300	5·57	92	0·217	41 600	37 500
4·93	170	0·429	52 400	87 400	5·62	103	0·243	41 300	33 000
4·98	213	0·491	56 800	92 700	5·67	114	0·273	40 300	32 300
5·03	271	0·569	61 700	87 600	5·72	126	0·305	39 500	48 600
5·08	341	0·669	65 400	83 900	5·77	146	0·342	40 500	61 800
5·13	426	0·793	68 200	112 200	5·82	176	0·386	42 900	89 300
5·17	540	0·918	74 200		5·87	230	0·441	48 600	105 400
					5·92	316	0·516	56 600	93 800
					5·94	354	0·552	59 100	
	Average:		58 000	89 000		Average:		45 000	65 000

TABLE 42. — COMMERCIAL APPLE PECTIN, II.

($M_s = 67\,000$)

Medium: 0·2 N-NaCl. $\alpha = 140 \cdot 10^{-5}$. 7 200 R.P.M.

$c = 0{\cdot}53$ p. c.					$c = 0{\cdot}32$ p. c.				
x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
4·71	53	0·298	31 200	79 700	5·44	28	0·166	21 900	132 800
4·76	64	0·322	34 100	93 200	5·49	40	0·178	27 800	103 000
4·81	80	0·351	38 900	104 900	5·54	53	0·194	34 000	84 500
4·86	103	0·387	45 000	104 800	5·59	67	0·214	38 400	80 800
4·91	133	0·435	51 300	104 500	5·64	84	0·240	43 200	89 200
4·96	172	0·495	57 800	112 200	5·69	108	0·272	47 800	114 400
5·01	227	0·574	65 000	110 100	5·74	149	0·315	57 100	126 100
5·06	299	0·677	71 900	104 900	5·79	213	0·376	67 600	129 100
5·11	390	0·812	77 200	102 500	5·84	308	0·463	78 700	131 900
5·16	530	0·991	85 300	114 000	5·89	450	0·589	89 300	135 300
5·17	562	1·033	86 600		5·93	616	0·729	98 200	
	Average:		62 000	105 000		Average:		63 000	123 000

TABLE 43. — SODIUM PECTINATE FROM COMMERCIAL APPLE PECTIN.

($M_s = 58\,000$)

Medium: {0·20 M-NaCl, 0·03 M-Na_2PO_4, 0·02 M-KH_2PO_4} pH 7. $\alpha = 130 \cdot 10^{-5}$ 7 200 R.P.M.

$c = 0{\cdot}50$ p. c.					$c = 0{\cdot}25$ p. c.				
x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
4·74	80	0·343	37 400	28 500	5·47	54	0·160	39 300	3 950
4·79	86	0·372	36 700	28 600	5·52	55	0·176	36 100	7 030
4·84	93	0·403	36 100	35 000	5·57	57	0·192	33 700	6 970
4·89	101	0·436	36 000	33 400	5·62	58	0·209	31 500	28 500
4·94	110	0·473	35 800	41 600	5·67	63	0·227	31 200	47 900
4·99	121	0·513	35 900	49 400	5·72	72	0·246	32 700	59 700
5·04	137	0·558	37 000	78 800	5·77	85	0·269	34 900	68 800
5·09	166	0·611	40 600	122 000	5·82	106	0·297	38 900	116 800
5·14	223	0·678	48 700	247 000	5·87	146	0·334	47 300	134 600
5·17	318	0·735	63 700		5·92	211	0·386	58 900	179 000
					5·93	233	0·399	62 800	
		Average:	40 000	86 000			Average:	39 000	77 000

TABLE 44. — CITRUS PECTIN.

($M_s = 89\,000$)

Medium: 0·2 N-NaCl. $\alpha = 140 \cdot 10^{-5}$. 7 200 R.P.M.

$c = 0{\cdot}287$ p. c.					$c = 0{\cdot}191$ p. c.				
x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
4·70	36	0·134	47 800	65 000	5·44	28	0·072	50 400	36 300
4·75	42	0·149	49 700	91 100	5·49	31	0·081	49 200	54 700
4·80	52	0·167	54 300	80 600	5·54	36	0·092	49 800	73 700
4·85	63	0·190	57 100	89 200	5·59	44	0·106	52 500	88 500
4·90	78	0·217	61 300	90 500	5·64	56	0·122	57 500	107 300
4·95	97	0·251	65 300	115 000	5·69	75	0·144	64 600	126 500
5·00	128	0·295	72 500	152 900	5·74	106	0·173	75 400	128 300
5·05	185	0·356	86 000	187 300	5·79	151	0·216	85 200	151 900
5·10	291	0·449	106 300	204 400	5·84	230	0·278	100 000	203 700
5·15	479	0·599	129 800	187 600	5·89	405	0·383	126 700	173 900
5·17	576	0·681	136 800		5·94	660	0·558	140 500	
		Average:	86 000	159 000			Average:	93 000	156 000

TABLE 45. — BEET PECTIN.

($M_s = 62\,000$)

Medium: 0·2 N-NaCl. $\alpha = 140 \cdot 10^{-5}$. 7 200 R.P.M.

$c = 0{\cdot}30$ p. c.					$c = 0{\cdot}50$ p. c.				
x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
4·72	34	0·177	27 400		5·47	61	0·331	19 100	
				80 600					56 300
4·77	41	0·189	30 700		5·52	71	0·348	20 900	
				76 000					57 800
4·82	49	0·203	33 700		5·57	83	0·368	22 900	
				86 100					56 900
4·87	60	0·219	37 900		5·62	97	0·392	24 900	
				94 200					78 400
4·92	75	0·240	42 800		5·67	120	0·420	28 500	
				134 500					111 000
4·97	103	0·268	52 100		5·72	167	0·457	35 100	
				173 100					127 400
5·02	155	0·308	67 500		5·77	229	0·508	44 300	
				185 300					145 900
5·07	241	0·370	86 500		5·82	341	0·582	57 000	
				166 600					131 600
5·12	360	0·463	102 200		5·87	490	0·691	68 500	
				122 400					122 900
5·17	486	0·594	106 600		5·92	690	0·845	78 200	
				77 800					120 800
5·18	517	0·625	107 500		5·94	790	0·922	81 700	
	Average:		69 000	136 000		Average:		43 000	117 000

TABLE 46. — ALGINIC ACID.

($M_s = 50\,000$)

Medium: 0·2 N-Na_2CO_3. $\alpha = 115 \cdot 10^{-5}$. 6 600 R.P.M.

$c = 0{\cdot}304$ p. c.				
x	Z	c_i	$(M_i)_w$	$(M_i)_z$
4·74	47	0·176	70 900	
				17 400
4·79	49	0·199	64 800	
				26 500
4·84	52	0·223	60 600	
				24 800
4·89	55	0·249	56 800	
				49 900
4·94	61	0·277	56 100	
				67 400
4·99	70	0·308	57 300	
				108 800
5·04	87	0·346	62 800	
				157 000
5·09	119	0·395	74 500	
				187 100
5·14	173	0·466	90 900	
				207 400
5·17	223	0·523	103 900	
		Average:	69 000	119 000

TABLE 47. — SODIUM GUM.
($M_s = 256\,000$)

Medium: 0·2 N-NaCl, pH 5·8. $\alpha = 147 \cdot 10^{-5}$. 3 600 R.P.M.

$c = 0{\cdot}245$ p. c.					$c = 0.491$ p. c.				
x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
4·70	54	0·081	484 000		5·45	83	0·187	234 000	
4·75	56	0·101	399 000	49 000	5·50	104	0·217	250 000	364 000
4·80	62	0·123	358 000	176 000	5·55	130	0·253	266 000	356 000
4·85	69	0·148	328 000	183 000	5·60	159	0·298	274 000	317 000
4·90	83	0·176	328 000	329 000	5·65	194	0·354	279 000	310 000
4·95	102	0·210	335 000	364 000	5·70	240	0·421	287 000	330 000
5·00	130	0·253	351 000	429 000	5·75	305	0·507	301 000	371 000
5·05	179	0·311	389 000	565 000	5·80	400	0·617	321 000	417 000
5·10	265	0·393	451 000	691 000	5·85	514	0·760	332 000	382 000
5·15	390	0·515	502 000	674 000	5·90	643	0·940	333 000	336 000
5·18	475	0·612	511 000	567 000	5·94	765	1·116	331 000	324 000
		Average:	412 000	516 000			Average:	307 000	352 000

TABLE 48. — SODIUM GUM.
($M_s = 256\,000$)

Medium: {0·10 M-NaCl, 0·06 M-Na_2HPO_4, 0·04 M-KH_2PO_4} pH 7. $\alpha = 147 \cdot 10^{-5}$. 3 000 R.P.M.

$c = 0{\cdot}120$ p. c.					$c = 0{\cdot}240$ p. c.				
x	Z	c_i	$(M_i)_w$	$(M_i)_z$	x	Z	c_i	$(M_i)_w$	$(M_i)_z$
4·71	17	0·061	244 000		5·45	48	0·112	273 000	
4·76	20	0·067	259 000	428 000	5·50	55	0·125	278 000	309 000
4·81	24	0·074	279 000	478 000	5·55	64	0·141	284 000	344 000
4·86	30	0·082	311 000	587 000	5·60	75	0·159	292 000	357 000
4·91	38	0·093	343 000	618 000	5·65	90	0·180	307 000	411 000
4·96	47	0·106	369 000	546 000	5·70	112	0·206	331 000	494 000
5·01	62	0·123	416 000	714 000	5·75	142	0·239	358 000	533 000
5·06	85	0·145	478 000	809 000	5·80	187	0·282	396 000	617 000
5·11	123	0·178	558 000	944 000	5·85	248	0·339	434 000	627 000
5·16	168	0·223	603 000	785 000	5·90	335	0·415	475 000	663 000
5·19	198	0·257	613 000	682 000	5·95	456	0·517	514 000	676 000
		Average:	448 000	733 000			Average:	389 000	582 000

Gum arabic was investigated as sodium gum in order to avoid the risk of autohydrolysis. This substance is more sensitive to the action of hydrogen ions than are the pectins and alginic acid.

The weight-average molecular weight M_w for the acidic pectins agrees rather well with the molecular weight M_s determined from sedimentation velocity and diffusion. The measurements on two samples purified separately (I and II) of the commercial apple pectin give an idea of the degree of reproducibility in the determinations.

Sodium pectinate exhibits a lower value of M_w than that obtained for the acid substance, just as was the case in the determinations of M_s. The difference by sedimentation equilibrium is the larger one, however.

It should be pointed out that the $(M_i)_w$-value in some cases, curiously enough, passes through a minimum (Tables 41, 43, 46). This fact indicates the possibility that the substance is acted upon by forces other than those normally determining the equilibrium, forces due, for instance, to the charges, after all. Anomalies in the sedimentation equlibrium of high molecular carbohydrates are often ascribed to a swelling pressure. Such a pressure would give rise to a maximum in the series of $(M_i)_w$-values, however, and is not likely to be active here. In any case, where there is a minimum, the difference between M_w and M_s is most marked as might be inferred from the tables.

The values of M_w obtained for sodium gum are considerably higher than the M_s-value. This is a rather surprising fact that can hardly be due to either swelling or charges. It is difficult to give any reasonable explanation of this fact unless we may assume some kind of aggregation.

POLYDISPERSITY.

'At the present time it appears that the most satisfactory manner of characterizing the molecular weight of polydisperse macromolecular materials having markedly non-spherical molecules in solution is in terms of the weight-average molecular weight and the non-uniformity coefficient β' (Kraemer, UC, p. 352).

Though there may be, in addition, other methods for characterizing polydispersity (cf. p. 56) we might agree that this property becomes most obviously manifest from the molecular weight distribution in a properly estabilished sedimentation equilibrium.

TABLE 49. — NON-UNIFORMITY COEFFICIENTS.

Preparation	Medium	Conc. per cent	β
Commercial apple pectin, I . .	0·2 N-NaCl	0·50 0·30	0·93 0·86
Commercial apple pectin, II . .	» »	0·53 0·32	1·03 1·16
Sodium pectinate from commercial apple pectin	0·20 M-NaCl, 0·03 M-Na_2HPO_4, 0·02 M-KH_2PO_4, pH 7	0·50 0·25	1·24 1·17
Citrus pectin	0·2 N-NaCl	0·287 0·191	1·11 1·02
Beet pectin	» »	0·50 0·30	1·00 1·16
Alginic acid	0·2 N-Na_2CO_3	0·304	1·04
Sodium gum, I	0·2 N-NaCl	0·491 0·245	0·52 0·67
Sodium gum, II	0·10 M-NaCl, 0·06 M-Na_2HPO_4, 0·04 M-KH_2PO_4, pH 7	0·240 0·120	0·90 0·99

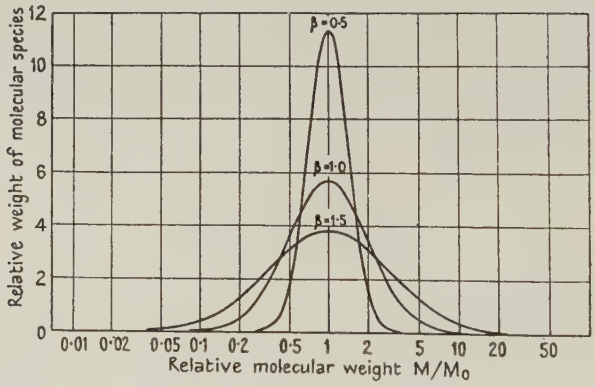

Fig. 29.—Effect of the non-uniformity coefficient β on the logarithmic distribution function. (Lansing and Kraemer, 108.)

Table 49 gives the non-uniformity coefficient for the various type substances. The significance of the β-value is pictured by the curves in Fig. 29 (Lansing and Kraemer, 107).

As may be seen the variation of β for the various pectin preparations is not very large, sodium pectinate probably being polydisperse to a somewhat higher degree than the acidic substances. This fact may be connected with the decrease observed in both M_s and M_w on neutralizing the acidic pectin.

The β-values for the pectins and alginic acid are of the same magnitude as those reported for samples of cellulose acetate, and methyl cellulose (UC, p. 353).

The two preparations of sodium gum differ markedly in polydispersity. The pretreatment (precipitation, time for dialyzing the samples free from hydrochloric acid etc.) may be responsible for this fact. The possibility should also be taken into account that the change of medium brings about a change in the strength of the aggregating forces postulated above, with a result that the degree of polydispersity changes, just as was presumed above to be the case when apple pectin is transferred into a neutral solution.

SUMMARY.

1. Three types of acid polyuronides, viz. pectic substances, alginic acid and gum arabic, have been investigated.

2. The preparation of the substances has been described and the percentage of uronic acid anhydride and methoxyl groups for the various preparations has been estimated.

3. Some of the substances were selected as type substances and subjected to a closer examination.

4. The viscosity of solutions of the polyuronides in various media has been measured and the results discussed.

5. The jellying properties of the type pectins have been studied by means of a 'jelly strength tester' constructed for this purpose.

The apparatus and the measuring process have been described.

6. The acid polyuronides are colloidal electrolytes. Data concerning their conductivity and electrophoretic mobility have been collected with a view to throwing some light on the 'charge effects' met with in several measurements, particularly where pure water is used as a medium.

7. The sedimentation of the polyuronides under various conditions has been illustrated. The sedimentation velocity in pure water is strongly reduced as a result of the influence of the charges. Evidence has been given pointing to the possibility that the sedimentation velocity of the particles within the boundary range is reduced to a higher degree than that of the particles in the bulk of the solution, because of the action of the strong diffusion potential set up at the boundary.

8. The diffusion velocity of the polyuronides in pure water is abnormally high, due to the action of a very strong diffusion potential. The possible influence of the diffusion potential upon the sedimentation of the particles was discussed.

Evidence has been given showing that the frictional forces acting in sedimentation and diffusion respectively differ to some extent, except, perhaps, for the lowest concentrations.

9. Values of the partical specific volume for the various polyuronides have been determined and discussed.

10. Various molecular constants have been determined from sedimentation and diffusion data.

Extracted pectins and alginic acid were found to have molecular weights of the order of 50 000—90 000, while the free pectins may have particles four times as large.

Gum arabic was found to have particles of the order of 250 000—300 000, varying with the acidity of the medium.

From measurements of streaming birefringence it is evident that the particles in solution are very elongated, even the gum particles. The length and the thickness of the particles have been calculated by means of formulae deduced from the assumption of ellipsoidal, non-hydrated particles. To judge from these calculations it seems probable that the pectin particles at least are made up of single chain molecules. Too much importance should not be attached to such a statement, however, as the particles dealt with here are no doubt hydrated, a fact the importance of which in this connection is at present unknown.

11. Additional information about the molecular size of the polyuronides has been gained from sedimentation equilibrium measurements. In most cases the average molecular weight M_w obtained by this method agrees rather well with the value M_s calculated from sedimentation and diffusion data. In some cases, however, there are discrepancies, the reasons for which are not clear. The M_w-values for gum arabic, especially, were found to be considerably higher than the M_s-value.

The polydispersity of the various polyuronides was characterized by means of the non-uniformity coefficient β.

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